THE EFFECTS OF NERVE STIMULATION ON PACEMAKING ACTIVITIES OF BIOLOGICAL TISSUES

THESIS

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by

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PACEMAKING TISSUES

INTRODUCTION

The demonstration that increasing inhibitory frequency to physiological pacemakers can, under certain circumstances, produce the response usually associated with excitation (Perkel et al, 1964; Reid, 1969; Levy et al, 1970; Dong and Reitz, 1970), prompted further investigation into the phenomenon. In this chapter a general description is given of physiological pacemakers, their occurrence, nature, mechanism and responses.

Pacemaker cells generate spike potentials spontaneously. Usually a collection of such cells is required to excite neighbouring cells and thus act as a physiological pacemaker. Pacemaker cells are muscle or nerve cells.

Some of the tissues which contain pacemakers and hence show physiological pacemaking activities are smooth muscle in the gastro-intestinal tract, ureter, uterus and some of the blood vessels (Speden, 1970); cardiac muscle, particularly that in the sinoatrial node, and some of the cells in the central nervous system.

The smooth muscle of the uterus shows pacemaking activity only if it is oestrogen-primed (Marshall, 1962). Thus, the uterine smooth muscle from an immature or ovariectomised animal is non-pacemaking and becomes pacemaking with the administration of oestrogen to such an animal. It is not known how oestrogen converts the non-pacemaking uterine muscle into a pacemaking one; however oestrogen has been shown to increase the membrane potential of the myometrial cell from an average of -35 mV in the inactive uterus to an average of about -57 mV in the oestrogen-primed, spontaneously active uterus (Marshall, 1962).

Spontaneous spike discharges have been recorded from many neurones in the central nervous system (Huttenlocher, 1961;

Hubel, 1959; Eccles, 1973). It is technically difficult to determine the dependence of these neurones for their spontaneous discharge on the excitatory input from other neurones, and ultimately from the stimulation of various sense organs. In fact it is likely that the majority of the neurones are dependent upon the excitatory input from other neurones for their spontaneous discharges (Burns, 1958), though there are some e.g. Purkinje cells in the cerebellum (Eccles, 1973), which do not require excitatory influence for their spontaneous spike discharges.

The pacemaking activities of gut smooth muscle, cardiac muscle and respiratory neurones will now be discussed as their investigation formed the basis of the present thesis.

SMOOTH MUSCLE

Bozler (1941) has classified smooth muscle into two groups — multi-unit and unitary. The multi-unit smooth muscle is similar to skeletal muscle in that it is composed of motor units and contracts only if activated extrinsically by nerves or hormones. It is not active spontaneously. Examples of this type of smooth muscle are found in the nictating membrane, the iris, ciliary body, urinary bladder and most of the blood vessels.

The unitary smooth muscle demonstrates pacemaking activities. Examples of this type of smooth muscle, as mentioned previously, are found in the gastro-intestinal tract, ureter, uterus and some types of blood vessels. The unitary smooth muscle that has been studied extensively is that found in the guinea-pig taenia coli and cat jejunum.

The following is a brief and simplified description of the electrophysiological activity of the unitary type of smooth muscle:

In the guinea-pig taenia coli Na^+, K^+ and Cl^- are not passively distributed across the smooth muscle membrane. Na^+ are actively transported out of the smooth muscle cell and K^+ and

Cl^- into the muscle cell (Casteels, 1971). The equilibrium potentials of Na^+, K^+ and Cl^- for the smooth muscle cell calculated by Nernst Equation from their intra- and extracellular concentrations are +52 mV, -89 mV and -24 mV respectively (Casteels, 1969). Casteels introduced the intra- and extracellular concentrations of these ions for the guinea-pig taenia coli, and their permeability constants, into the Goldman Equation and obtained a resting diffusion potential of -37 mV. However the average measured resting membrane potential of these cells is -55 mV (Bulbring and Kuriyama, 1963(a)). This discrepancy between the measured and calculated membrane potential may be due to an electrogenic extrusion of Na^+ from the smooth muscle cell (Casteels, 1969). Hence the resting membrane potential of the smooth muscle cell of the guinea-pig taenia coli is probably due partly to a diffusion potential and partly to an electrogenic extrusion of Na^+ from the cell.

The membrane potential of the smooth muscle cells of the cat jejunum undergoes slow spontaneous depolarization and repolarization and these changes have been called slow waves. It has been suggested that these slow waves are due to changes in the activity of the electrogenic sodium pump which may depend on the cyclical production of ATP by mitochondria (Job, 1969). When ATP production is increased the electrogenic sodium pump is more active and the smooth muscle cells becomes repolarized; and when decreased the cell becomes depolarized.

Job (1971) in a subsequent paper proposed that the intestinal slow waves were not due to cyclical production of ATP by mitochondria, since altering the rate of mitochondrial ATP synthesis by antibiotics such as Valinomycin and Monensin did not alter the frequency of slow waves, though their amplitude was altered. However there is no evidence to show that the antibiotics he used altered the frequency of cyclical production of ATP by mitochondria although they did alter the mitochondrial

ATP synthesis rate (Pressman et al, 1967). Job (1971) postulated that the depolarization phase of the intestinal slow waves occurred as a result of accumulation of ATP at the smooth muscle cell membrane, which increased the permeability of the membrane to Na^+; consequently there was a passive flux of Na^+ into the muscle cell, which stimulated the electrogenic sodium pump, increasing the Na^+ efflux during depolarization. Only when ATP at the cell membrane was depleted because of the activity of the electrogenic sodium pump would the permeability of the cell membrane to Na^+ decrease and the cell become repolarized. According to this postulation the maximal Na^+ efflux would occur during depolarization, whereas Job (1969) had shown that the maximal Na^+ efflux occurred at the beginning of the repolarization phase of slow waves. Furthermore, the frequency of the slow waves would be determined by the time required for ATP to reach a threshold concentration and this would be dependent on mitochondrial ATP synthesis rate. If this were so, one would have expected that the antibiotics which alter the mitochondrial ATP synthesis rate would alter the frequency of the slow waves, but this was not so (Job, 1971).

When a smooth muscle cell becomes depolarized to a threshold voltage an action or spike potential is initiated. The frequency of smooth muscle action potentials depends on the degree of depolarization - the greater the degree of depolarization the higher the frequency of action potentials. In this respect the smooth muscle cell behaves like the slow stretch receptor of the crayfish (Eyzaguirre and Kuffler, 1955) where there is a similar relationship between generator potential and the frequency of action potentials.

In some smooth muscle cells, in addition to the above two types of changes in the membrane potential a third type may be seen. This is the "pacemaker" potential which is a slow depolarization of the smooth muscle cell culminating in an action potential. The pacemaker potentials are often recorded from

smooth muscle cells where the action potentials arise from the slow waves.

In other smooth muscle cells, although there may be slow waves, the action potential arises because of spread of excitation from neighbouring discharging cells. In this instance there is usually no relationship between the slow waves and action potentials. The ionic mechanisms for the slow waves and the action potentials in smooth muscle cells are different and they probably arise from different molecular parts of the cell membrane (Tamai and Prosser, 1966). The slow waves are probably due to changes in the rate of electrogenic extrusion of Na^+ (Job, 1969). On the other hand the action potentials in the smooth muscle cells of the guinea-pig taenia coli (Brading et al, 1969) and cat jejunum (Liu et al, 1969) are mainly due to the influx of Ca^{2+}.

Contraction of smooth muscle is dependent on its action potential and the higher the frequency of action potentials the stronger the contraction of the smooth muscle.

To summarise the events in smooth muscle:

depolarization of smooth muscle cell (slow waves) $\longrightarrow$ pacemaker potentials $\longrightarrow$ action potentials $\longrightarrow$ contraction.

These mechanisms have a number of variations in smooth muscles from different organs and different species.

The activity of smooth muscle is altered by mechanical stretch, changes in external ionic concentrations, humoral factors and neural activity.

Mechanical stretching of the intestinal smooth muscle causes depolarization of the smooth muscle cells resulting in initiation or an increase in the frequency of their action potentials (Bulbring, 1955). This initiates or augments smooth muscle contraction. The mechanisms by which mechanical stretching causes depolarization of the smooth muscle is not known; it is not due to release of acetylcholine from nerve terminals in the smooth

muscle, since the response is not blocked by atropine (Bulbring, 1955).

The smooth muscle cells of guinea-pig taenia coli are depolarized and their frequency of action potentials increase when the $[K^+]$ in the extracellular fluid ($[K^+]_o$) is increased. The relationship between the membrane potential and the logarithm of $[K^+]_o$ is linear when the $[K^+]$ is above 30 mM — a depolarization of 38 mV per tenfold increase in $[K^+]_o$ — and non-linear when the $[K^+]_o$ is below 30 mM (Kuriyama, 1963).

On the other hand when the concentration of Ca^{2+} in the solution bathing the guinea-pig taenia coli is increased above the normal physiological levels the smooth muscle cells are hyperpolarized and their spontaneous spike discharges, though transiently increased sometimes, are always finally slowed or abolished (Bulbring and Kuriyama, 1963(a)). The hyperpolarization of the smooth muscle cells in excess $[Ca^{2+}]_o$ is associated with an increase in their membrane conductance, probably to K^+ (Bulbring and Tomita, 1969(c)). Reduction in external $[Ca^{2+}]$ below the normal physiological level depolarizes the smooth muscle cells and this is associated with a decrease in the membrane conductance, probably to K^+. However, when the external $[Ca^{2+}]$ is further reduced to zero the smooth muscle cells are further depolarized and this is associated with a high membrane conductance, probably to Na^+ (Bulbring and Tomita, 1969(c)).

Amongst the humoral factors affecting the gut smooth muscle are adrenaline, noradrenaline and acetylcholine. Adrenaline causes relaxation of the intestinal smooth muscle. The mechanism of action of adrenaline is complex. Adrenaline causes an increase in the conductance of the smooth muscle cell membrane of the guinea-pig taenia coli to K^+ and Cl^- (Bulbring and Tomita, 1969(a); Ohashi, 1971). Consequently the smooth muscle cells hyperpolarize, their frequency of spike discharges decreases and relaxation of the smooth muscle occurs.

This effect of adrenaline on smooth muscle membrane conductance to K^+ and Cl^- is blocked by phentolamine (α-adrenergic blocker). However, in the presence of phentolamine, adrenaline still blocks the spike discharges by suppressing the pacemaker potentials (Bulbring and Tomita, 1969(b)) and this effect is reduced or abolished by propranolol (a β-adrenergic blocker).

Hence the inhibitory action of adrenaline on the smooth muscle of the guinea-pig taenia coli is in part brought about by hyperpolarization due to increased K^+ and Cl^- conductance of the muscle membrane (α-effect) and in part by suppression of the pacemaker potential (β-effect).

The inhibitory action of adrenaline on the guinea-pig taenia coli is potentiated by an increase in the external $[Ca^{2+}]$ and is diminished by a reduction in the external $[Ca^{2+}]$ (Bulbring and Tomita, 1969(c)). Hence it has been suggested that adrenaline might increase the Ca^{2+}-binding in the membrane which would increase its K^+-permeability (α-action) and it might also suppress removal of Ca^{2+} from the binding site in the membrane (β-action). Removal of Ca^{2+} would decrease K^+-conductance and cause depolarization (pacemaker potential); this would be prevented by adrenaline. The process of Ca^{2+}-binding in and Ca^{2+} removal from the membrane may depend on metabolic energy supply and it has been shown that there is a significant increase in ATP and creatine-phosphate content of the smooth muscle coinciding with the physiological action of adrenaline (Bueding et al, 1967).

It has also been suggested that adrenaline may cause hyperpolarization of the intestinal smooth muscle cells by increasing the activity of the electrogenic sodium pump, so that there is an extrusion of positively charged sodium ions from the muscle cells (Burnstock, 1958). This mechanism of action of adrenaline in causing hyperpolarization of the smooth muscle cells is of minor importance (Bulbring and Tomita, 1969(a)).

While adrenaline causes relaxation of intestinal

smooth muscle it may cause contraction of smooth muscle in other sites e.g. cutaneous blood vessels.

The action of noradrenaline on the intestinal smooth muscle is similar to that of adrenaline, though it is less potent. However, the actions of adrenaline and noradrenaline are not always similar; their actions may be antagonistic e.g. adrenaline causes dilatation whereas noradrenaline causes constriction of skeletal muscle blood vessels.

Acetylcholine depolarizes the smooth muscle cells by a non-selective increase in the permeability of their membranes to all the ions (Bulbring and Kuriyama, 1963(b)). This initiates or increases the frequency of their spike discharges, leading to initiation or augmentation of smooth muscle contraction.

Stimulation of parasympathetic, sympathetic or intrinsic nerve alters the activity of the intestinal smooth muscle. The parasympathetic nerve is excitatory or motor to the intestinal smooth muscle (Garry and Gillespie, 1955). The transmitter substance released from the postganglionic parasympathetic nerve terminals is acetylcholine (Patton, 1965) and the mechanism of action of acetylcholine has been described above.

The sympathetic nerve is inhibitory to the intestinal smooth muscle (Garry and Gillespie, 1955). The transmitter substance released from the postganglionic sympathetic nerve terminals is noradrenaline and/or adrenaline (Patton, 1965) and their mechanism of action has been described above.

However Burn (1968) has reported that the sympathetic fibres are motor to the small intestinal muscle in the newborn rabbit and only in the first 1-2 weeks of post-natal life do the sympathetic fibres become inhibitory. He observed that the motor response is blocked by hyoscine and is thus cholinergic. On this basis he has suggested that the initially cholinergic sympathetic fibre becomes adrenergic when it develops the ability

to take up phenylalanine which is then converted into noradrenaline; but the cholinergic link in the sympathetic adrenergic transmission persists in the adult.

It has been shown that in addition to sympathetic nerves there are intrinsic or enteric nerves which are also inhibitory to the intestinal smooth muscle. The transmitter substance released from the enteric nerve terminals is said to be ATP and hence these enteric nerves are purinergic (Burnstock, 1972). The transmitter substance released from the purinergic nerve acts by producing a specific increase in conductance of the smooth muscle to K^+ only.

CARDIAC MUSCLE

For the initiation of an action potential in a cardiac muscle cell the membrane potential must be reduced to a threshold voltage. Once this threshold voltage has been reached there is a regenerative increase in the permeability of the cell membrane to Na^+, leading to a large flux of Na^+ into the cell and causing the rising phase of the cardiac action potential. The entry of Ca^{2+} into the cell also contributes in part to the rising phase of the cardiac action potential.

The cardiac cell is repolarized when there is a sudden increase in the permeability of the cell membrane to K^+, leading to a large efflux of K^+. Between the rising phase of the cardiac action potential and the repolarization phase there is a plateau phase of variable duration resulting from a slightly raised permeability of the cell membrane to Na^+ and a low permeability of the cell membrane to K^+.

The cells in the myocardium differ in the way the threshold voltage for the initiation of cardiac potential is reached in the physiologically intact heart. In one group of cells, the pacemaker cells, the threshold voltage is reached spontaneously, while in the other cells, the "driven" cells, the

threshold voltage is reached because there is current spread from the pacemaker cells. In the physiologically intact heart the pacemaking cells which generate action potentials at the fastest rate are normally found in the sinoatrial (S-A) node and it is from these cells that the action potentials spread to the rest of the heart. Hence the S-A node normally determines the heart rate and is thus called the pacemaker of the heart. Pacemaker cells are also found else-where in the heart e.g. atrioventricular node and Purkinje fibres; but these normally have a slower rate than the S-A node and therefore do not normally act as pacemakers of the heart.

The characteristic feature of the pacemaker cells is that they undergo spontaneous progressive depolarization during diastole until the threshold voltage is reached. This depolarization during diastole results in the pacemaker potential. On the other hand the "driven" cells do not depolarize during diastole, or if they do, their rate is so slow that, before their threshold is reached spontaneously, they are excited by current spread from neighbouring cells.

The progressive depolarization in diastole in pacemaker cells is due primarily to a time—dependent reduction in the potassium conductance of the cell membrane while the sodium conductance remains unaltered (Vassalle, 1966). Consequently the Na^+ current going into the cell exceeds the K^+ current leaving the cell, resulting in depolarization of the cell. Just before the threshold voltage is reached there is also a voltage-dependent increase in sodium conductance of the cell membrane.

The frequency at which the action potentials are generated by the pacemaker cells in the S-A node will depend on how soon after the membrane has been repolarized the threshold voltage for the initiation of action potential is reached. This will depend on the membrane potential at the beginning of diastole, the rate of diastolic depolarization and the threshold

voltage (Hoffman, 1967).

These can be altered by neural and humoral activity and by changes in the extracellular concentrations of ions.

Cardiac vagal efferent discharge causes bradycardia by decreasing the frequency of action potentials initiated by the pacemaker cells of the S-A node. This vagal action results from hyperpolarization of the pacemaker cells and a decrease in the rate of diastolic depolarization (Hoffman, 1967). The ionic basis for both these effects of vagal action is an increase in the potassium conductance of the cell membrane of the pacemaker cell (Trautwein, 1963). This is in fact the action of acetylcholine - the neurotransmitter released from the post-ganglionic vagal terminals (Patton, 1965).

Cardiac sympathetic activity increases the frequency of action potentials initiated by the S-A node and hence causes tachycardia. This sympathetic effect results from an increase only in the rate of diastolic depolarization of the pacemaker cells; the level of maximum polarization of the pacemaker cells during diastole is not altered (Hutter and Trautwein, 1956). The increase in the rate of the diastolic depolarization is due to the potentiation of the time-dependent reduction in the potassium conductance of the pacemaker cell membrane. This is the action of noradrenaline, the neurotransmitter released from the post-ganglionic sympathetic nerve terminals (Ganong, 1971).

Thus it would appear that stimulation of the vagus nerve would cause bradycardia, and increasing the frequency of stimulation of the vagus nerve, should cause further slowing of the heart rate. On the other hand, stimulation of the cardiac sympathetic nerves should cause tachycardia, and increasing the frequency of stimulation of the cardiac sympathetic nerve should cause further increase in the heart rate.

The humoral factors which affect the S-A node include

noradrenaline, adrenaline and acetylcholine. Noradrenaline and adrenaline increase the S-A firing rate, their mechanisms of action being similar. The mechanism of action of noradrenaline has been described above. The catecholamines acting on the S-A node not only come from the sympathetic nerve terminals in the S-A node, but also from the adrenal medulla and possibly from sympathetic nerve terminals elsewhere in the body. On the other hand all the acetylcholine acting on the S-A node is normally released by the vagal terminals in the S-A node. However, if acetylcholine is injected intravenously it causes a transient bradycardia.

Changes in the external concentrations of K^+ and Ca^{2+} affect the S-A discharge rate. If the $[K^+]_o$ is increased above the normal physiological level the S-A rate is increased (Lu, 1970; Hashimoto et al, 1970). This has been shown to be due to the maximal diastolic depolarization of the pacemaker cells in the S-A node becoming less negative and the increase in the S-A rate occurs despite a reduction in the rate of diastolic depolarization of the pacemaker cells (Lu, 1970). A decrease in $[K^+]_o$ below the normal physiological level decreases the S-A rate. This has been shown to be due to a reduction in the rate of diastolic depolarization of the pacemaker cells; the level of maximal diastolic potential and the threshold potential are not altered (Lu, 1970). Toda and West (1967) however, have reported that the S-A rate is unaffected when the $[K^+]_o$ is varied, even though an increase in the $[K^+]_o$ makes the maximal diastolic potential of the pacemaker cells less negative.

An increase in $[Ca^{2+}]_o$ has been shown to increase the rate of diastolic depolarization of the pacemaker cells and to make their threshold potential and maximal diastolic potential less negative (Seifen et al, 1964). The change in the S-A rate due to changing the $[Ca^{2+}]_o$ depends on the relative magnitude of these actions. Seifen et al. (1964) have shown that when the

$[Ca^{2+}]_o$ is increased from 0.3 mM to 6.3 mM the S-A rate increases. This is due to the effect on the S-A rate of increase in the rate of diastolic depolarization and the reduction in the maximal diastolic potential being greater than the effect of reduction of threshold potential. When the $[Ca^{2+}]_o$ is increased above 6.3 mM the S-A rate decreases since the effect of decrease in the threshold potential prevails over the effect of increase in the rate of diastolic depolarization and the reduction of maximal diastolic potential (Seifen et al, 1964).

RESPIRATORY NEURONES

There are neurones in the brain stem which produce periodic bursts of action potentials in phase with either inspiration or expiration. These neurones are termed respiratory neurones and they collectively constitute the respiratory centre. It is believed that the respiratory neurones discharging during inspiration ultimately produce inspiratory motorneurone discharges and those discharging during expiration ultimately produce expiratory motorneurone discharges.

Various hypotheses have been put forward to account for the periodic bursts of action potentials of these respiratory neurones. One hypothesis is that the neurones in the respiratory centre discharge spontaneously and continuously and the periodicity is imposed upon them by extrinsic inhibition provided by vagal afferent discharges from the pulmonary stretch receptors and by pontine pneumotaxic mechanisms (Pitts et al, 1939).

Another hypothesis is that the respiratory centre is fundamentally quiescent and requires excitation from other sources. Evidence in support of this hypothesis is that the intracellular recordings from the respiratory neurones show that action potentials were brought about by temporal summation of excitatory postsynaptic potentials; there were no slow oscillations of the membrane potential accounting for the firing of the

respiratory neurones (Salmoiraghi, 1963). It is the interplay of the external excitatory influence together with increasing firing threshold of the neurones and reciprocal innervation of the inspiratory and expiratory neurones that bring about the periodic discharge of the respiratory neurones.

The factors affecting the activity of the respiratory neurones include neural input from many sources e.g. pulmonary stretch receptors, central and peripheral chemoreceptors, cerebral cortex, pons and probably joint receptors. Hence an important way in which the respiratory neurones differ from the S-A node is that their activity is modified by neural input from many sources whereas the S-A node's activity is modified by neural input from sympathetic and parasympathetic nerves.

PARADOXICAL RESPONSES OF PACEMAKERS TO INCREASING INHIBITORY INPUT

One would expect that increasing the frequency of neural inhibitory input to a pacemaker would progressively slow its frequency of spike discharge. However there are theoretical grounds for believing that this may not be true under all circumstances. Perkel et al (1964) have shown with a mathematical model that during a progressive increase in the frequency of inhibitory input to a pacemaker there would occur periods or zones in which an increase in the frequency of inhibitory input to the pacemaker would produce an increase in the frequency of its spike discharge; this was called a "paradoxical" effect. The paradoxical effect only occurred if the inhibitory impulses were regularly spaced; if the delay caused by the inhibitory input was dependent upon the time at which the inhibitory input was applied in relation to the previous spike and if the slope between the two was positive and between 0 and 2. In these paradoxical zones the ratio of frequency of inhibitory input to frequency of pacemaker spike discharge is a simple one e.g. 1:2, 1:1, 2:1, etc. Outside the paradoxical zones an increase in the frequency of inhibitory input to a pacemaker will produce the normally expected progressive slowing of frequency of its spike discharge.

Perkel et al (1964) then went on to show that these predictions of "paradoxical zones" applied to abdominal ganglia of Aplysia and the stretch receptor of crayfish i.e. during a progressive increase in the frequency of inhibitory input to these two sets of neurones there were periods during which an increase in the frequency of the inhibitory input caused an increase in their rate of spike discharges.

These observations on the paradoxical response of the neurones in Aplysia and crayfish were extended to the sinoatrial node in the cat and rat by Reid (1969). Reid investigated the response of the sinoatrial node in the cat and rat to vagal stimulation. He showed that on increasing the frequency of stimulation of the vagus nerves there occurred paradoxical zones in which increasing frequency of vagal stimulation produced an acceleration of the pacemaking activity of the sinoatrial node. In these paradoxical zones there was a simple ratio of pacemaker rate to vagal stimulation rate e.g. 2:1, 3:2, 1:1, 2:3, 1:2, and 1:3. Outside the paradoxical zones the orthodox response was obtained i.e. increasing the frequency of stimulation of the vagus nerves produced a progressive slowing of the heart rate.

Levy et al (1969) and Dong and Reitz (1970) obtained similar paradoxical responses in the dog i.e. an increase in the heart rate despite increasing frequency of vagal stimulation.

PURPOSE OF THIS STUDY

Investigation of three types of pacemaking tissues forms the basis of the present thesis - the sinoatrial node in the cat and man, the smooth muscle in rabbit colon and the respiratory neurones in man. The purpose common to all investigations was the elucidation of responses by pacemakers to inhibitory input.

The first series of experiments was done on cats to determine:-

1. the effects on the pacemaking activities of the sinoatrial node of the arrival of a single vagal volley at

different times during the cardiac cycle; and

2. the duration of the effects of a single vagal volley on the pacemaking activities of the sinoatrial node.

An important consideration in undertaking this particular series of experiments was to examine more closely the phenomenon of paradoxical responses by the sinoatrial node.

The second series of experiments was done on the rabbit distal colon to answer the following questions:-

1. Is there a paradoxical response of the smooth muscle in the distal colon to increasing frequency of inhibitory stimulation rate? and

2. Is there a positive motor response of the smooth muscle in the distal colon to the stimulation of sympathetic nerves in the newborn rabbit, or is the response an inhibition as in the mature animal?

The third series of experiments was done on human subjects to see whether paradoxical responses of pacemaking tissues to increasing inhibitory input could be obtained under normal physiological circumstances. During the immediate post-exercise recovery period there is both an increasing inhibitory input and a decline of excitatory input to the sinoatrial node and the respiratory neurones. Hence by observing the heart rate and minute ventilation in the immediate post-exercise recovery period one can monitor the responses of the sinoatrial node and the respiratory neurones to the increasing inhibitory input and decreasing excitatory input. The effects of both humoral and neural excitatory input to the sinoatrial node could be blocked by a β-adrenergic blocker, leaving only increasing inhibitory input to the sinoatrial node during the post-exercise recovery period. However the excitatory and inhibitory inputs to the respiratory neurones is far more complex and it is not possible to block the excitatory input to the respiratory neurones by drugs.

The third series of experiments therefore consisted

of recording the heart rate and minute ventilation during the post-exercise recovery period in control and β -adrenergic blocked individuals. The heart rate and minute ventilation were also recorded before and during the period of exercise.

CHAPTER II

EFFECTS OF VAGAL STIMULATION ON THE PACEMAKING ACTIVITIES OF THE SINOATRIAL NODE OF THE CAT

1. RESPONSES OF THE SINOATRIAL NODE TO THE STIMULATION OF VAGUS NERVES AT DIFFERENT TIMES DURING THE CARDIAC CYCLE

EXPERIMENTAL METHOD

Twelve experiments were done on 11 cats. The cats were anaesthetised with sodium pentobarbital, 30 mg/kg, given intravenously. Tracheal cannulae were inserted and both vagi were exposed in the neck. The cats were then artificially ventilated through their tracheostomy and the chests opened by splitting the sternum in the midline. Both stellate ganglia were identified and excised to minimise changes in the sympathetic effects on the sinoatrial node (Levy et al., 1966). The vagi in the neck were divided and the peripheral ends of both vagi were placed on platinum electrodes. Both the vagi were stimulated synchronously with single supramaximal electrical shocks - 10 to 15 V with a pulse duration of 2 msec - once every 6 to 10 seconds. Grass stimulator S48 and Grass isolation unit SIU 5 were used to deliver electrical shocks.

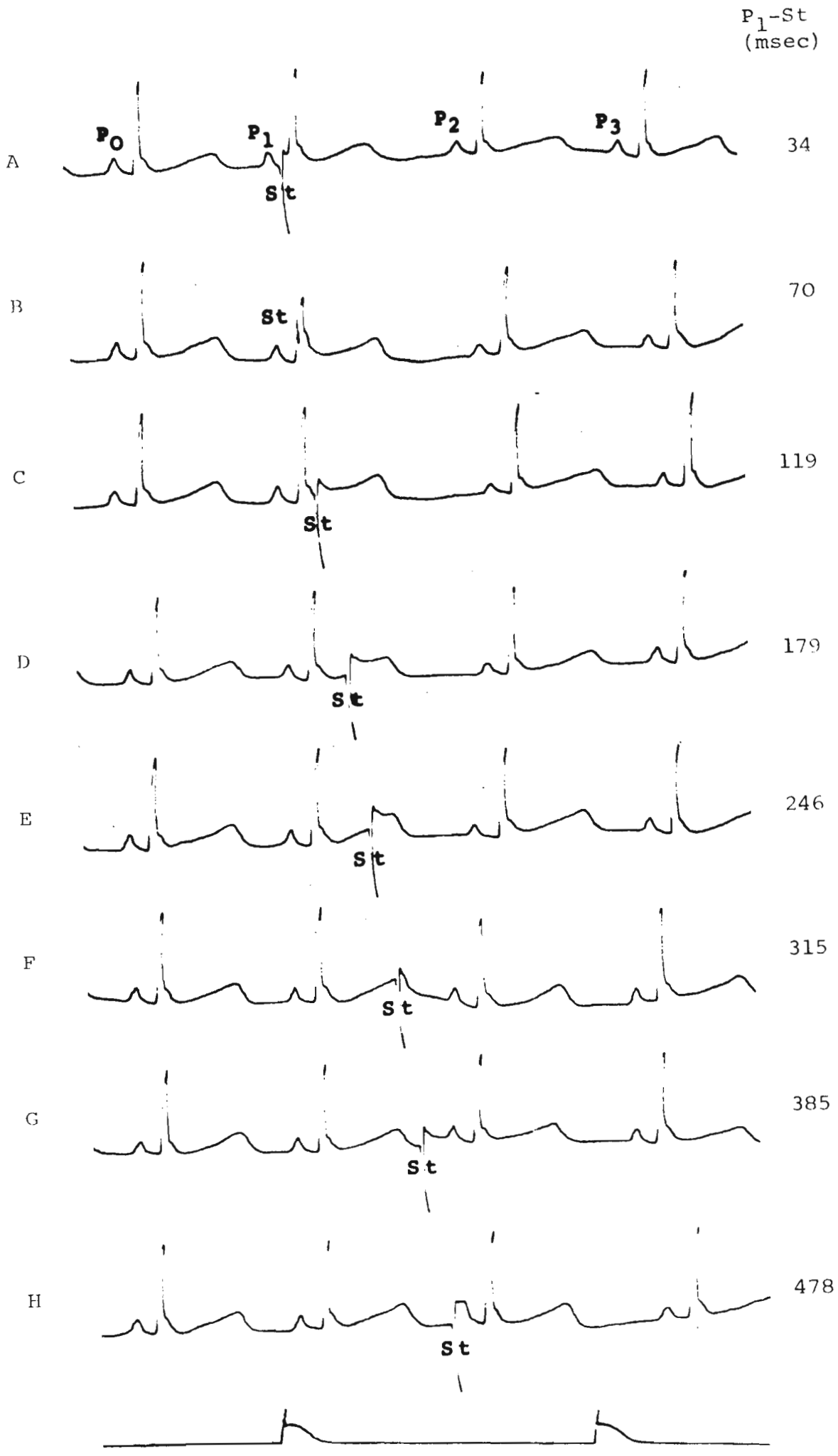
An electrocardiogram, using standard leads I or II, was recorded on an Ediswan direct writing recorder, the paper speed being 6 cm/sec. The error in the paper speed was less than 1%.

The vagi were stimulated with single shocks 50 to 100 times and as no attempt was made to stimulate the vagi at any particular point during the cardiac cycle, the stimuli fell randomly throughout the cardiac cycle.

METHOD OF ANALYSIS

Figure 1 is excerpts from a record obtained during an experiment. It shows the effects of stimulating the vagus nerves at progressively longer intervals after a P wave.

The P waves were sequentially labelled P_0, P_1, P_2 and P_3 (refer to Fig. 1). Stimulus artefact was labelled St.


P1-St
(msec)
A
B
C
D
E
F
G
H
P0
P1
P2
P3
St
34
70
119
179
246
315
385
478

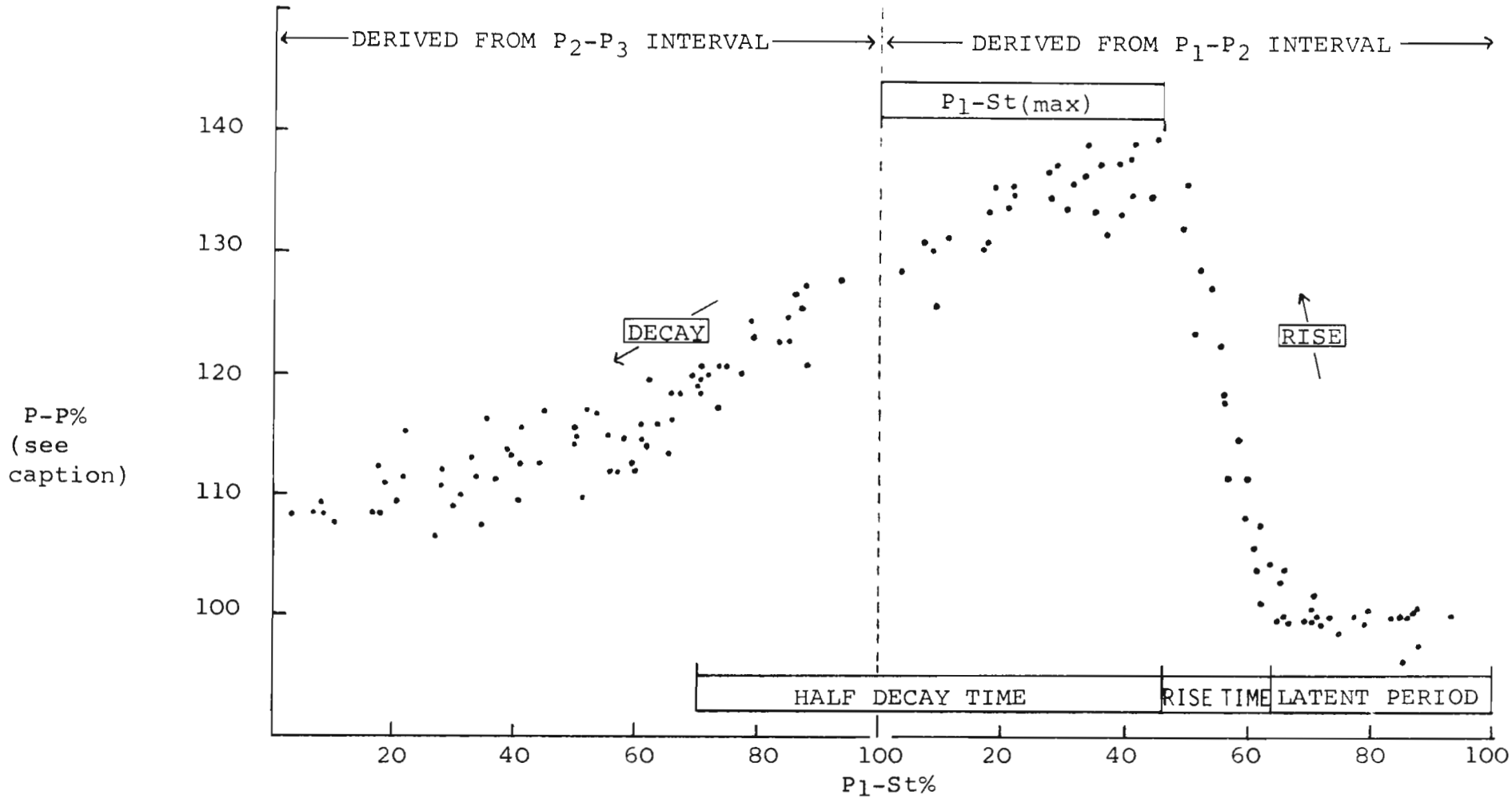


Fig. 2. Effects on P_1-P_2% and P_2-P_3% of stimulating vagus nerves at different times during the P_1-P_2 interval in a single experiment. The points to the right of the broken line show the effects on the P_1-P_2 intervals and the points to the left on the P_2-P_3 intervals. Each point represents the result of a single stimulation of vagus nerves. Y AXIS: P-P interval expressed as a percentage of P_0-P_1; P_1-P_2% to the right and P_2-P_3 to the left of the broken line. For this experiment the mean P_0-P_1 was 461 msec.

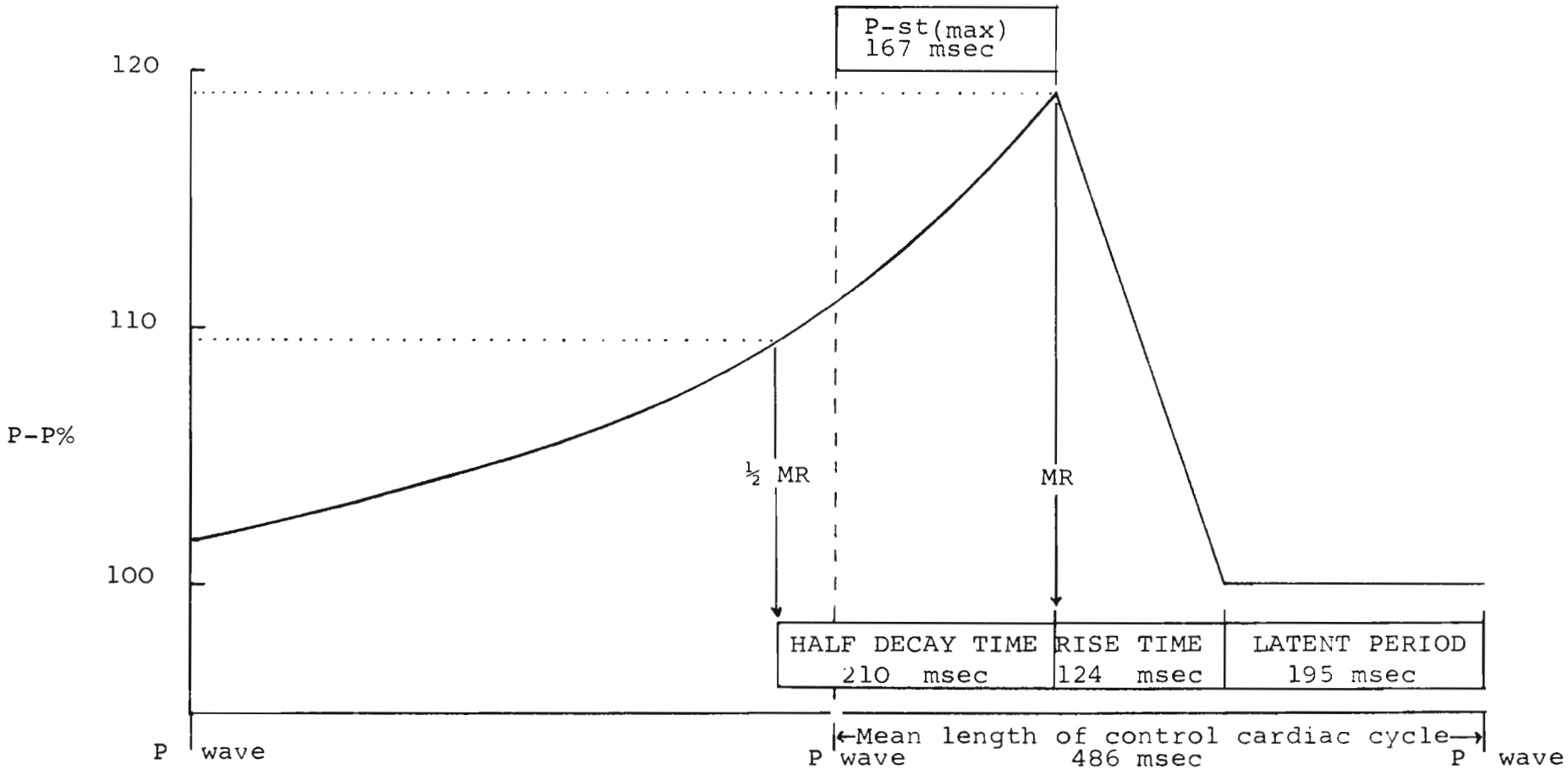


Fig. 3. Mean results plotted graphically. Curve to the right of the broken line shows the effects on P_1-P_2% and that to the left on P_2-P_3% when the vagi were stimulated during P_1-P_2. MR = maximum response. ½ MR = half-maximum response.

Measurements (correct to within 0.01 cm) of P_1-St, P_0-P_1, P_1-P_2 and P_2-P_3 intervals were made from the peaks of P waves. The vagal stimulation always occurred between P_1 and P_2. P_0-P_1 was taken as the control interval and P_1-St, P_1-P_2 and P_2-P_3 were expressed as a percentage of P_0-P_1 and thus labelled P_1-St%, P_1-P_2% and P_2-P_3%. P_1-P_2% showed the effect of a single electrical stimulation of vagus nerves on the duration of the first cardiac cycle after vagal stimulation. Similarly P_2-P_3% showed the effect of vagal stimulation on the duration of the second cardiac cycle following vagal stimulation.

This method of analysis was employed for every single stimulation of the vagus nerves throughout each experiment.

A graph (Fig. 2) showing the effects of vagal stimulation in a single experiment, on P_1-P_2% and P_2-P_3% at different times during the P_1-P_2 interval was plotted. From Fig. 2 an estimate was made of the P_1-St% interval at which the prolongation of P_1-P_2 due to vagal stimulation, was maximal. This value was converted into absolute time by multiplying it by the mean duration of P_0-P_1 for any particular experiment. This will be referred to as P_1-$St_{(max)}$. Furthermore, the minimum P_1-St% interval at which vagal stimulation did not have an effect on the timing of P_2 was read from Figure 2. This was subtracted from 100 and the remainder, multiplied by the mean duration of P_0-P_1, gave the period prior to the peak of the P_2 wave during which stimulation of the vagus nerves had no effect on the timing of P_2. This was termed latent period.

The latent period plus P_1-$St_{(max)}$ was subtracted from the mean P_0-P_1 interval. This was termed rise time.

An estimate of the magnitude of the maximal response to vagal stimulation was made from the same figure. Finally an estimate was made of the half-decay time from Fig. 2, i.e. the time interval between vagal stimulation having maximal effect and vagal stimulation having half the maximal effect.

The slope of the positive part of the curve (Fig. 3) was computed between the x co-ordinates at which maximum and

half-maximum response occurred. Subtracting P_0-P_1 (msec) from the P-P interval (msec) at P_1-$St_{(max)}$ and dividing it by two gives the y co-ordinate. This is divided by the half-decay time (x co-ordinate) to give the slope.

RESULTS

The data obtained from the 12 experiments performed on 11 cats are tabulated in Table I. Table II shows the means, standard deviations (SD), range and standard error (SE) obtained from the data included in Table I. Figure 3 shows these mean results plotted graphically.

The effect of vagal stimulation depended upon when, during the P-P interval, the vagi were stimulated (Fig. 3). When the vagi were stimulated 167 msec (SD±64) after the peak of P_1 there was maximum prolongation of P_1-P_2. The effects progressively decreased when the P_1-St interval increased from 167 msec (SD±64) to 291 msec (SD±70). When P_1-St interval was more than 291 msec (SD±70) it had no effect on the duration of P_1-P_2. Similarly the effects decreased when the P_1-St interval decreased from 167 msec (SD±64) to zero; however, even when the vagal stimulation occurred at the peak of P_1 it still had considerable effect in prolonging P_1-P_2. The duration of P_2-P_3 was also prolonged when the vagi were stimulated during P_1-P_2 (Fig. 3) and in this instance the prolongation of the cardiac cycle increased progressively as the vagi were stimulated later and later during P_1-P_2. The slope of the curve is positive at P_1-St intervals of less than 167 msec (SD±64) and negative at P_1-St intervals between 167 msec (SD±64) and 291 msec (±70). The positive slope ranged from 0.13 to 0.48 with a mean of 0.23 (SD±0.09).

If one supposes that the P_1-P_2 interval were unaffected by the stimulus, P_1-P_2 would be the same length as P_0-P_1, and hence one could anticipate the arrival of P_2. When considering the effects of vagal stimulation on the pacemaker potentials, the results are clarified by relating timing of the stimulus

TABLE I

EFFECTS OF VAGAL STIMULATION ON THE PACEMAKING ACTIVITIES OF THE S-A NODE

Expt. No.	P_O-P_1 (msec)	P_1-$St_{(max)}$ (msec)	Latent period (msec)	Magnitude of maximum response % of P_O-P_1	Rise time (msec)	Half-decay time (msec)	Slope of positive part of the curve
1	360	90	180	125	90	180	0.25
2	496	149	223	113	124	124	0.26
3	448	112	188	115	148	139	0.24
4	390	109	176	110	105	148	0.13
5	517	140	181	109	196	140	0.17
6	513	190	180	112	143	190	0.16
7	579	261	203	120	115	434	0.13
8	704	296	268	121	140	155	0.48
9	445	111	200	110	134	134	0.17
10	461	212	166	137	83	350	0.24
11	497	174	224	130	99	323	0.23
12	416	154	150	127	112	200	0.28

P_O-P_1 is the mean duration of the cardiac cycle.

P_1-$St_{(max)}$ is the interval after P_1 when the arrival of the vagal stimulus produced a maximum response. Magnitude of maximum response is the duration of the maximally prolonged cardiac cycle expressed as a percentage of P_O-P_1.

TABLE II

THE MEANS, STANDARD DEVIATIONS (SD), STANDARD ERRORS OF THE MEANS (SE) AND RANGES OF EFFECTS OF VAGAL STIMULATION ON THE PACEMAKING ACTIVITIES OF THE S-A NODE

	P_O-P_1 (msec)	P_1-$St_{(max)}$ (msec)	Latent period (msec)	Magnitude of maximum response % of P_O-P_1	Rise time (msec)	Half-decay time (msec)	Slope of positive part of the curve
MEAN	486	167	195	119	124	210	0.23
SD	91	64	32	9	31	102	0.09
SE	26	18	9	2.6	9	29	0.03
RANGE	360-704	90-296	150-268	109-137	83-196	124-434	0.13-0.48

to this anticipated arrival time of P_2. To do this the time intervals mentioned above are subtracted from the control cardiac cycle lengths. The vagal stimulation had no influence on the timing of P_2 when the vagi were stimulated at 195 msec (SD±32) or less, before the anticipated P_2. The effects then progressively increased as the interval between St and the anticipated P_2 increased from 195 msec (SD±32) to 319 msec (SD±47). As the interval between St and the anticipated P_2 was further increased from 319 msec (SD±47) to 486 msec (SD±91), the latter being the duration of the control cardiac cycle, the effects decreased progressively, though even at 486 msec (SD±91) the effects were still considerable.

The half-decay time i.e. the time from the peak effect of vagal stimulation to the time vagal stimulation had only half the maximal effect was 210 msec (SD±102).

From these observations the latent period was 195 msec (SD±32) and the rise time was 124 msec (SD±31).

P_1-$St_{(max)}$ depended upon the cardiac cycle length (Fig. 4). The relationship between the two is described by the equation:

$$y = 0.62\ x - 131 \text{ msec}$$

where $y = P_1\text{-}St_{(max)}$ and x = cardiac cycle length (P_0-P_1). Thus P_1-$St_{(max)}$ increased significantly ($p < 0.001$) with longer cardiac cycle lengths.

Similarly a significant correlation ($p < 0.01$) was noted between the duration of the latent period and the duration of cardiac cycle. The latent period increased with longer cycle lengths (Fig. 5). The equation

$$y = 0.27\ x + 62 \text{ msec}$$

where y = latent period and x = cardiac cycle length, describes the relationship between the latent period and the cardiac cycle length.

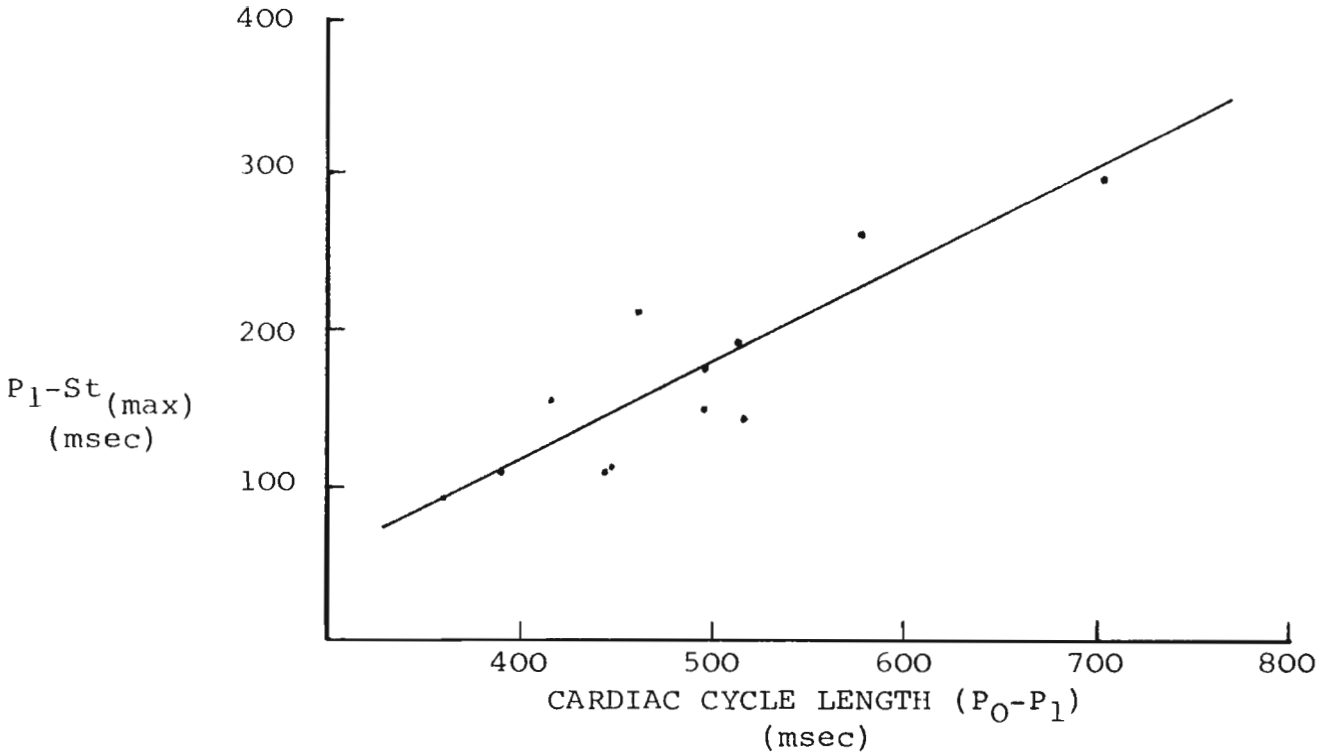


Fig. 4. P_1-$St_{(max)}$ for different cardiac cycle lengths. Each point represents the result of a single experiment. The straight line curve is the linear regression line computed for these points.

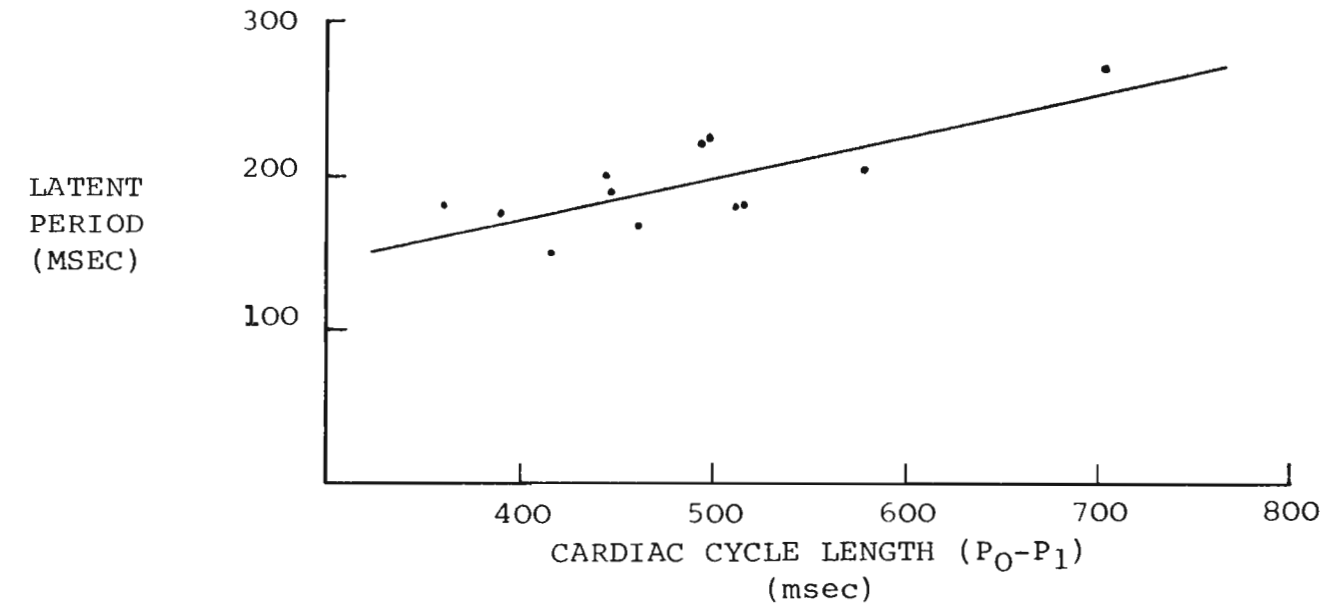
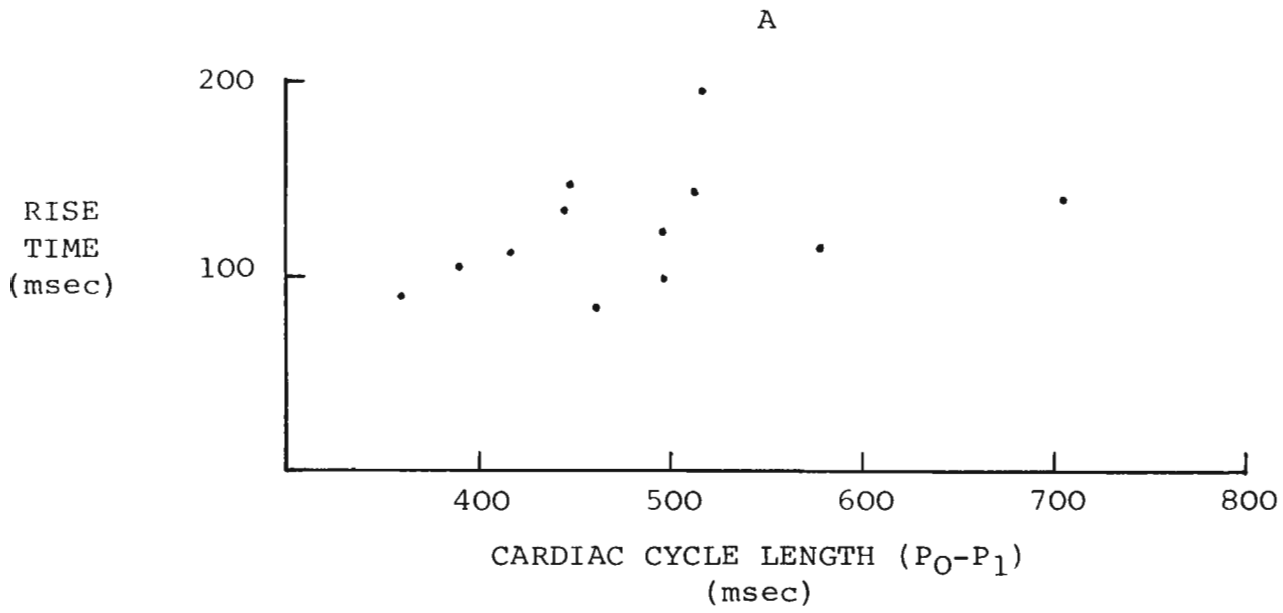


Fig. 5. Latent period of the effects of vagal stimulation at different cardiac cycle lengths. Each point represents the result of a single experiment. The straight line is the linear regression line computed for these points.



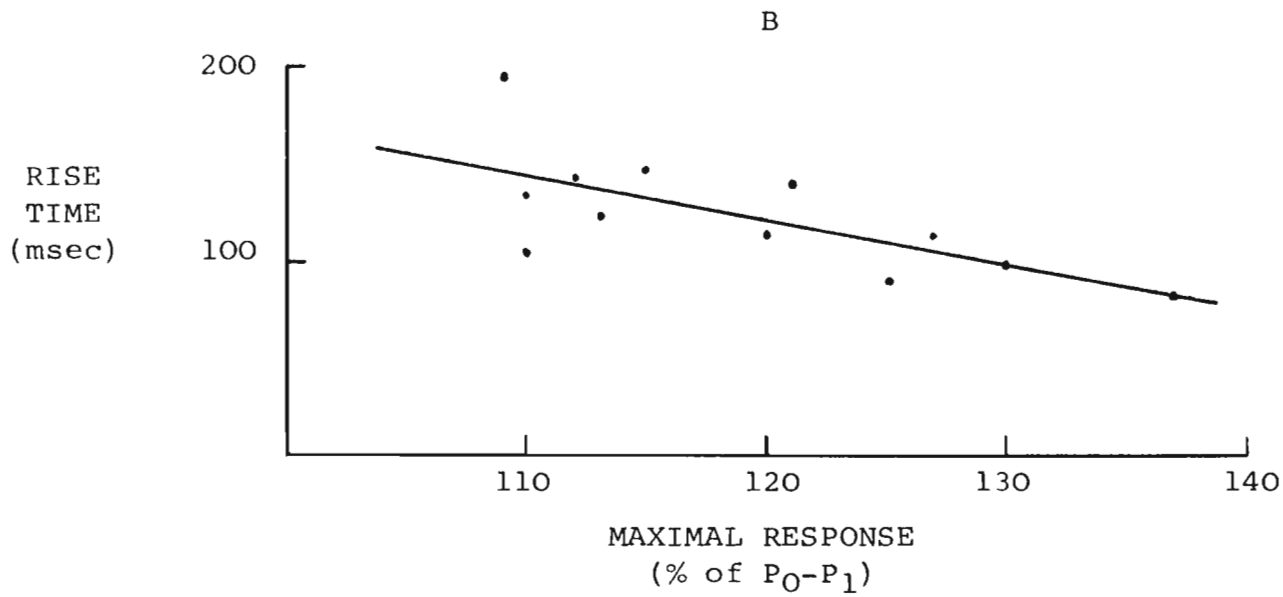


Fig. 6. A. Rise time of vagal response at different cardiac cycle lengths. Each point represents the result of a single experiment.

B. Graph showing the inverse relationship between rise time and maximal response to vagal stimulation. Each point represents the result of a single experiment. The straight line is the linear regression line computed for these points.

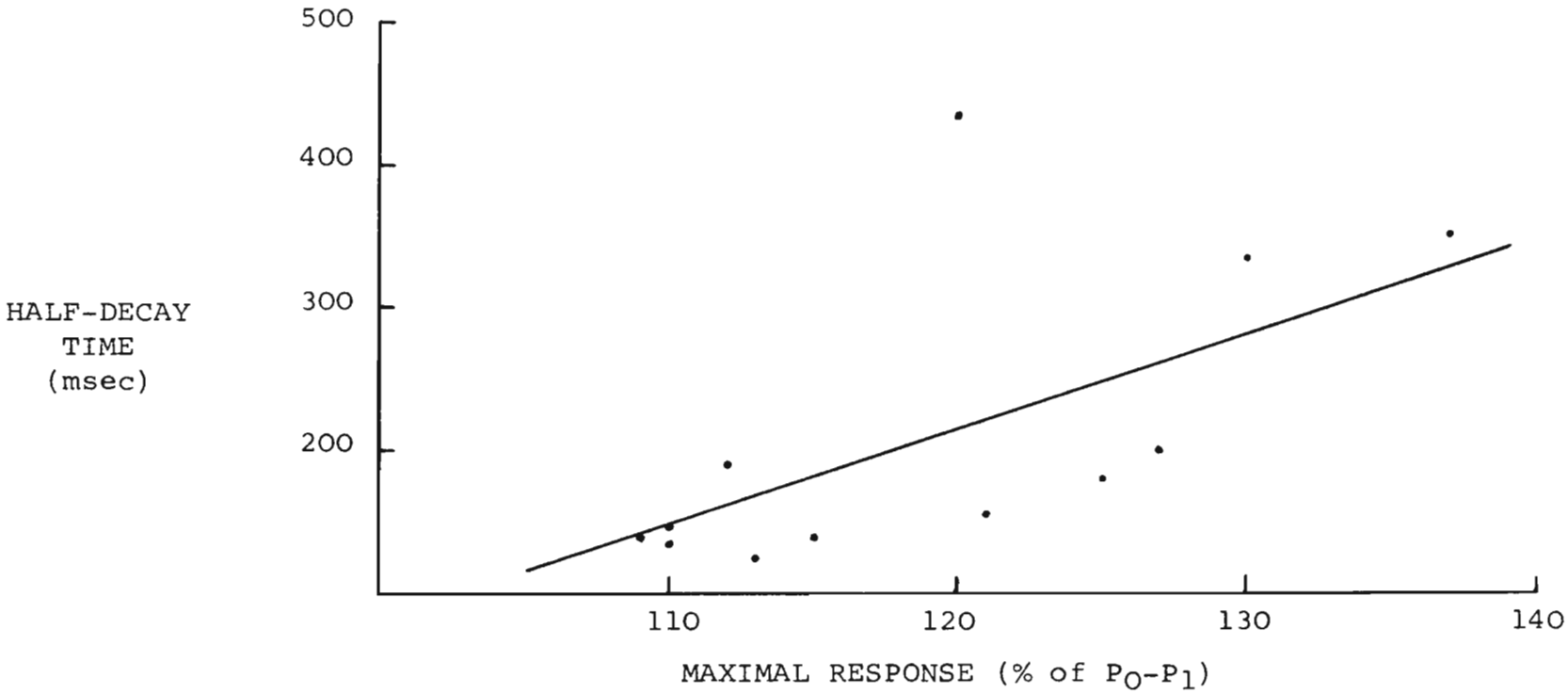


Fig. 7. Graph showing the relationship between half-decay time and maximal response to vagal stimulation. Each point represents the result of a single experiment. The straight line is the linear regression line computed for these points.

The rise time was not significantly correlated ($p > 0.1$) with the length of the cardiac cycle (Fig. 6A). However, the magnitude of the maximal response to vagal stimulation was inversely proportional to the rise time ($p < 0.02$; Fig. 6B) and the equation

$$y = -2.27\ x + 394 \text{ msec}$$

where y = rise time and x = maximal response, describes the relationship between the two.

The half-decay time was 210 msec (SD$\pm$102). It was significantly correlated ($p < 0.02$) with the magnitude of the maximum response to vagal stimulation and the relationship between the two is described by the equation:

$$y = 6.58\ x - 573 \text{ msec}$$

where y = half-decay time and x = magnitude of the maximum response (Fig. 7).

The half-decay time was not significantly correlated with either the cardiac cycle length ($p > 0.5$) or the rise time ($p > 0.1$).

DISCUSSION

Brown and Eccles (1934) found that the latent period for the effects of vagal stimulation on the sinoatrial (S-A) node was between 100 and 160 msec in 12 cats, whereas in 2 cats where the cardiac cycle lengths were 500 msec or longer, the latent period was about 200 msec. However these authors found no correlation between the duration of latent period and the duration of cardiac cycle when the cardiac cycle lengths were below 500 msec. They estimated that 20 msec of this latent period of between 100 and 160 msec was taken up by the conduction of the impulse in the vagus nerves, by the transmission across the autonomic ganglia, and by the conduction in the postganglionic vagal fibres to their terminations in S-A node. The remainder of the latent period was attributed to the release time of

acetylcholine from the postganglionic vagal terminals, its diffusion time to the pacemaker cells and in its action on the pacemaker cells.

Levy et al (1970) found that in dogs the mean latent period was 199 msec. They estimated the transmission time in the vagus nerves to be 30-40 msec; this included the synaptic delay in autonomic ganglia and transmission in postganglionic fibres. These authors suggested that the remainder of the latent period included acetylcholine release time from nerve endings, diffusion time to the pacemaker cells and the delay from the time of arrival of acetylcholine to the actual response of the pacemaker cells.

Dong and Reitz (1970) also found that the latent period in dogs was approximately 200 msec for the effects of vagal stimulation on S-A node.

The mean latent period in the present series of experiments in cats was 195 msec (SD±32). The range was 150-268 msec and the standard error of the mean was 9 msec (Table II).

As described by Brown and Eccles (1934) and Levy et al. (1970) the latent period in the present series includes:

impulse conduction time in the vagus nerve;
synaptic delay at the autonomic ganglia;
impulse conduction time in the postganglionic vagal fibres; and
synaptic delay at the S-A node.

The term synaptic delay at the S-A node includes acetylcholine release time from postganglionic vagal terminals, its diffusion time and its interaction with receptors in the S-A node resulting in altered membrane permeability of the pacemaker cells.

Since the above authors have measured the latent period in their experiments with reference to the beginning of

the P waves, the conduction time from the pacemaker cells in S-A node to the atria must also be included in the latent period. There are no figures available for conduction time in the S-A node of either cats or dogs. However, in rabbits the conduction of impulses in the S-A node has been shown to be slow - less than 0.05 m/sec and the rapid depolarization of pacemaker cells occurs 50-60 msec before that of atrial muscle fibres (de Carvalho et al, 1959). Similarly Toda and West (1967) found that the pacemaker cells in the S-A node of the rabbit depolarized on the average 40-60 msec in advance of atrial depolarization. The pacemaker cells may depolarize as long as 150 msec before the atrial muscle fibres (West, 1955). It is unlikely that the conduction time in the S-A node of the cat and dog differs markedly from that in the rabbit. The slow conduction time in the S-A node may be attributed to the fact that between P cells (of the S-A node) there are no intercalated discs and small gap junctions are rarely present (James et al, 1966).

In addition, in the present series of experiments the latent period was measured with reference to the peaks of P waves and not the beginning of the P waves. Hence the latent period in the present series will include the time from beginning to peak of P waves. This averaged 23 msec.

Thus the mean latent period of 195 msec in this series will be made up of:-

i) transmission time from the point of stimulation of vagus nerves in the neck to the postganglionic vagal terminals in the S-A node, which is estimated to be 20 msec (Brown and Eccles, 1934);
ii) synaptic delay at the S-A node;
iii) conduction time in the S-A node; and
iv) the time from beginning to peak of P waves - 23 msec.

Thus (i) and (iv) will account for 43 msec of the latent

period and the remaining 152 msec will be made up of synaptic delay at the S-A node and the transmission of the impulse in S-A node. If one assumes, following Toda and West, an average of 50 msec for the transmission time in the S-A node, the synaptic delay at the S-A node will be about 100 msec.

The latent periods in the present series of experiments in cats are longer than those determined by Brown and Eccles (1934). Thus a range of 127-245 msec in the present series (after making allowance for the time between the beginning and peaks of P waves) as compared with the range of 100-160 msec obtained by Brown and Eccles (1934) in 12 cats, and about 200 msec in 2 cats with cardiac cycle lengths of more than 500 msec. Unfortunately Brown and Eccles did not state the mean latent period.

However in the present series in 4 out of 12 experiments the cardiac cycle lengths were greater than 500 msec and in another 2 nearly 500 msec - 496 and 497 msec (Table I). This may account for the higher range obtained in the present series as compared with that obtained by Brown and Eccles (1934).

Brown and Eccles (1934) found no correlation between durations of latent periods and cardiac cycle lengths except when the cardiac cycle lengths were 500 msec or more. The present study clearly showed a correlation between the durations of latent periods and cardiac cycle lengths. No explanations can be offered for these differences. The longer latent periods at longer cardiac cycle lengths might have been due to slower conduction in the vagus nerves, longer synaptic delay at the autonomic ganglia, slower conduction in postganglionic nerve fibres, longer synaptic delay at the S-A node, slower conduction in the S-A node and longer interval between the beginning and peak of P waves. No attempt was made to investigate this any further.

Similar long latent periods and therefore probably long synaptic delays at the junction between postganglionic

nerve terminals and effector cells, have been found in guinea-pig taenia coli and cat submandibular gland. The latent period of the evoked excitatory junction potential in response to nerve stimulation in the guinea-pig taenia coli has been shown to be 100-200 msec (Bennett, 1966). The latent period for hyperpolarization of the secretory acinar cells in the submandibular gland of the cat in response to single shock stimulation of parasympathetic nerve was 180-350 msec (Creed and Wilson, 1969). It is very likely that the long latent periods in the guinea-pig taenia coli and cat submandibular gland are due to long synaptic delays at the junction between postganglionic nerve terminals and effector cells.

The synapses in the S-A node of the cat, the taenia coli of the guinea-pig and submandibular gland of the cat, all of which have long synaptic delays, are cholinergic and involve the interaction of acetylcholine with muscarinic receptors. On the other hand, at other cholinergic synapses which involve the interaction of acetylcholine with nicotinic receptors, the synaptic delays are comparatively short. Thus the synaptic delay at mammalian neuro-skeletal muscle junction has been shown to be 0.22 msec (Hubbard and Schmidt, 1963) and at autonomic ganglia in the superior cervical ganglion 2-6 msec (Eccles, 1935).

Therefore it seems that long synaptic delays are a feature of cholinergic synapses which involve interaction of acetylcholine with muscarinic receptors.

The effects of vagal stimulation on cardiac cycle lengths depended upon when during the cardiac cycle the vagi were stimulated. To consider why this is so one must look at the effects of vagal stimulation on the pacemaker cells in the S-A node. Stimulation of the vagi causes hyperpolarization of the pacemaker cells and decreases the rate or slope of their diastolic depolarization (Hoffman, 1967). Both these actions of vagal stimulation will delay the pacemaker cells reaching the threshold

voltage for the initiation of the action potential and hence prolong the cardiac cycle length.

The magnitude of hyperpolarization caused by vagal stimulation has been shown to depend upon the membrane potential of the pacemaker cells (Castillo and Katz, 1955). The more negative the membrane potential of the pacemaker cells the less will be the hyperpolarization of the pacemaker cells caused by vagal stimulation and vice versa. Hence the hyperpolarization of the pacemaker cells caused by vagal stimulation would be maximal when the effects begin just before the membrane potential had reached the threshold voltage, and least at the beginning of diastolic depolarization. Consequently, the delay in the onset of the next cardiac cycle due to hyperpolarization would be minimal if the hyperpolarizing effects of vagal stimulation occurred at the beginning of diastolic depolarization. The delay would increase as the hyperpolarization occurred progressively later during diastolic depolarization until it was maximal just before the threshold voltage was reached.

The second action of vagal stimulation on pacemaker cells is to decrease the slope of diastolic depolarization. Presumably the slope of diastolic depolarization would depend upon the concentration of acetylcholine at the synaptic region of the pacemaker cells. Hence the slope of diastolic depolarization would probably increase as the concentration of acetylcholine released from the vagal terminals is being reduced by the action of cholinesterase. Whether the effects on the slope of diastolic depolarization due to vagal stimulation is also dependent upon the membrane potential of the pacemaker cells is not known. Assuming that the slope of diastolic depolarization is not dependent upon the membrane potential of the pacemaker cells then one would expect that the longer the time during which the slope is decreased the greater would be the effect in delaying the pacemaker cell from reaching the threshold. This would be so even if the slope of diastolic depolarization is increasing due to a reduction in the concentration of acetylcholine.

Thus the effects of vagal stimulation in delaying the next pacemaker action potential would be maximal if the decrease in slope occurred right at the beginning of diastolic depolarization and minimal when it occurred just before the diastolic potential has reached the threshold voltage.

Thus the effects of vagal stimulation on cardiac cycle length would be dependent upon the additive effects of these two actions - hyperpolarization which would be most effective in prolonging the cardiac cycle length when it occurred just before the threshold voltage was reached and decrease in slope of diastolic depolarization which would be most effective when it started at the beginning of diastolic depolarization.

The vagal effect on the cardiac cycle length, when it began just before the threshold voltage was reached, would be primarily due to its hyperpolarization effect. When the vagal effects began at progressively longer intervals before the threshold voltage was reached the magnitude of the response to vagal stimulation increased initially. The most probable reason for this was that the effect on the cardiac cycle length due to hyperpolarization was diminishing at a slower rate than the effects due to change in slope of diastolic depolarization was increasing. The peak response to vagal stimulation would occur when the effect on cardiac cycle length due to hyperpolarization was decreasing at a rate equal to the rate of increase in effect due to the change in slope of diastolic depolarization. In the present series this occurred 124 msec (SD$\pm$31) before the pacemaker cells would have been normally expected to reach the threshold voltage. This value, as would be expected, coincides with rise time.

As the mean duration of the control cardiac cycle was 486 msec (SD$\pm$91) the duration of diastolic depolarization would be over 250 msec. Therefore the peak response to vagal stimulation must have occurred when the vagal effects began somewhere in the middle of diastolic depolarization and certainly not at the beginning.

When the effects on cardiac cycle length due to hyperpolarization began diminishing at a faster rate than the effects due to change in slope were increasing, the magnitude of the response to vagal stimulation would start to decrease.

When the vagal effects began during the previous pacemaker action potential, then by the time the pacemaker cell had repolarized and started to depolarize again (i.e. beginning of diastolic depolarization) some of the acetlycholine released from the vagal terminals would already have been destroyed and hence the decrease in slope at the beginning of diastolic depolarization would be less than if the effects had begun during the diastolic depolarization itself. This would account for a decreasing response to vagal stimulation when the effects started before the beginning of diastolic depolarization.

Levy et al (1970) ascribed the variable effects of vagal stimulation on the S-A node, depending upon when during the cardiac cycle the vagi were stimulated, solely to the duration of the decrease in slope of diastolic depolarization of the pacemaker cells. If this were so, the peak response to vagal stimulation would occur when the vagal effects i.e. hyperpolarization and decrease in slope, occurred at the very beginning of diastolic depolarization. This was not found to be so in the present study, nor do the experimental results of the above authors suggest this. In the experiments of Levy et al (1970) the elapsed time to peak response was 456 msec (mean) from the time of vagal stimulation. The mean latent period was 199 msec. Hence the peak response occurred when the vagal effects began 257 msec before the threshold for the initiation of action potential was reached. This would include the time by which the control cardiac cycle length was prolonged as a result of vagal stimulation. Since the mean control cardiac cycle length in their experiments was 414 msec and the peak response to vagal stimulation averaged 25.9% the cardiac cycle length must have been prolonged by 107 msec. Therefore the peak response must

have occurred when the vagal effects began 150 msec before the pacemaker cells would have been normally expected to reach their threshold voltage. This is comparable to a mean of 124 msec obtained in the present series. Clearly with a mean cardiac cycle length of 414 msec in their series of experiments the duration of diastolic depolarization would be much greater than 150 msec and therefore the peak response to vagal stimulation in their experiments could not have occurred when the vagal effects started at the beginning of diastolic depolarization.

The rise time in the present series was not significantly correlated ($p > 0.1$) with the length of the cardiac cycle (Fig. 6). If Levy et al's hypothesis were correct, i.e. the maximal response to vagal stimulation would occur when the vagal effects started at the beginning of diastolic depolarization of the pacemaker cells, then one would have expected a significant correlation between rise time and cardiac cycle length as the duration of diastolic depolarization varies with cardiac cycle length.

On the other hand there was a significant inverse correlation ($p < 0.02$) between the magnitude of maximal response to vagal stimulation and rise time (Fig. 6). If the rise time were short, the magnitude of maximal response was large and vice versa. Therefore when the magnitude of maximal response was large the decrease in slope of diastolic depolarization of pacemaker cells must have taken place over a shorter period before the expected threshold was reached, and when the maximal response was small the decrease in slope of diastolic depolarization must have taken place over a longer period. So, clearly the magnitude of the maximal response to vagal stimulation could not have been determined primarily by the decrease in slope of diastolic depolarization but by the amount of hyperpolarization produced by vagal stimulation.

There was a significant correlation ($p < 0.001$) between $P_1\text{-}St_{(max)}$ and cardiac cycle length ($P_0\text{-}P_1$). $P_1\text{-}St_{(max)}$ increased

with longer P_0-P_1, the ratio of increase in P_1-$St_{(max)}$ to increase in P_0-P_1 being 0.62 (see page 23). On the other hand the ratio of mean P_1-$St_{(max)}$ to mean P_0-P_1 was only 0.34. Hence with increasing P_0-P_1 the P_1-$St_{(max)}$ interval increased more rapidly than in a simple ratio of mean P_1-$St_{(max)}$ to mean P_0-P_1. This was to be expected from consideration of variation of rise time and latent period with different P_0-P_1 intervals. The P_1-$St_{(max)}$ interval is obtained by subtracting the rise time and the latent period from P_0-P_1. Rise time did not vary with different P_0-P_1 intervals. Latent period varied with different P_0-P_1 intervals, but the ratio of increase in latent period to increase in P_0-P_1 was 0.27, although the ratio of mean latent period to mean P_0-P_1 was 0.40. Therefore with increasing P_0-P_1 the P_1-$St_{(max)}$ interval would be expected to increase more rapidly than in a simple ratio of mean P_1-$St_{(max)}$ to mean P_0-P_1. From the experimental results this was found to be so.

The half-decay time was 210 msec (SD$\pm$102). It was significantly correlated ($p < 0.02$) with the magnitude of the response to vagal stimulation, but not with either the cardiac cycle length ($p > 0.5$) or the rise time ($p > 0.1$).

These results would explain the paradoxical responses of the S-A node to the vagal stimulation reported by Reid (1969), Levy et al (1970) and Dong and Reitz (1970). A paradoxical response to vagal inhibitory input would occur if the delay caused by the inhibitory input was dependent upon the time at which the inhibitory input was applied after the previous spike and if the slope between the two was positive and between 0 and 2 (Perkel et al, 1964).

In the present study the delay caused by the inhibitory vagal input was dependent upon when it was applied in relation to the previous P wave (and consequently the pacemaker action potential). The slope between the two was positive and ranged from 0.13 to 0.48 at all times when the vagi were stimulated during the P-P interval other than during rise time (rise time

being between P_1-St intervals of 167 msec (SD±64) and 291 msec (SD±70). Hence paradoxical responses of the S-A node to vagal stimulation occur and when they do the stimulation of the vagus nerves would be at P_1-St intervals other than during the rise time.

Furthermore during a paradoxical response the lowest vagal stimulation rate and sinoatrial rate would be when each vagal stimulus was positioned in the cardiac cycle where it elicited a maximal response and the highest rates when the vagal stimulus was positioned to produce the least response. Therefore as the frequency of vagal stimulation is increased during a paradoxical response the timing of the vagal volley in relation to the P wave would be expected to alter. At the lowest vagal stimulation rate the P_1-St interval would be short (167 msec, SD±64), and then as the rate increased the P_1-St interval would decrease until the stimulation of the vagus nerves in fact preceded the P waves. This has been reported by Reid (1969) and Levy et al (1970). Furthermore, the paradoxical zones (i.e. the stimulation frequency range over which there was an increase in the heart rate with increase in vagal stimulation frequency) would be longer if there were a marked response to vagal stimulation and the half-decay time was short.

2. DURATION OF EFFECTS OF VAGAL STIMULATION

A second series of experiments were done to determine the duration of effects of vagal stimulation on the pacemaking activities of the S-A node.

EXPERIMENTAL METHOD

The experiments were done on 8 cats. The method was as described previously. The vagus nerves were divided and both stellate ganglia were excised. The peripheral ends of both vagi were stimulated synchronously with single supramaximal shocks

every 6-8 seconds and a Standard Lead I or II electrocardiogram was recorded continuously. As no attempt was made to stimulate the vagi at any particular time during the cardiac cycle the stimuli fell randomly throughout the cycle.

In three of the experiments the procedure was different. The vagi were divided in the neck. The preganglionic fibres to the stellate ganglia were identified and cut and the stellate ganglia were stimulated with repetitive supramaximal electrical shocks, the repetition frequency being 50 Hz. In one experiment there was no response to repetitive stimulation and therefore the stellate ganglia were not stimulated with single shocks. In one of the remaining two, repetitive stimulation of the ganglia decreased the cardiac cycle length from 373 msec to 278 msec and in the other stimulation of the right stellate ganglion alone decreased the cardiac cycle from 429 msec to 339 msec. In these two the ganglia were subsequently stimulated with single supramaximal electrical shocks every 8 seconds. Thereafter the stellate ganglia in all three cats were excised and the peripheral ends of both vagi stimulated with single shocks, every 6-8 seconds, before and after intravenous administration first of propranolol (1 mg/kg — β-adrenergic blockade) and then of atropine (0.2 mg/kg - vagus blockade). Some of the cats were used for both this and the previous series of experiments.

METHOD OF ANALYSIS

The records from a single experiment were analysed as follows: the responses to 15 stimulations of the vagus nerves when the stimulation occurred early in the P-P interval (i.e. short P_1-St intervals) were measured. For each stimulation of the vagus nerves P_1-St, P_0-P_1 (the control cardiac cycle length) and the duration of 8-12 cardiac cycles subsequent to vagal stimulation were measured. The means of these measurements (i.e. of P_1-St, P_0-P_1, etc) were computed.

The time elapsed between vagal stimulation and a

TABLE III

THE MEAN RESPONSES OF 15 SINGLE STIMULATIONS OF THE VAGUS NERVES ON CARDIAC CYCLE LENGTHS IN A SINGLE EXPERIMENT

	Cardiac cycle after vagal stimulation	Duration of P-P interval (msec)	Time elapsed after vagal stimulation St-$P_{2 \ldots 12}$ interval (sec)	% of P_0-P_1	p
P_1-P_2	1st	500	0.404	120.8	<0.001
P_2-P_3	2nd	417	0.821	100.7	>0.01
P_3-P_4	3rd	432	1.253	104.3	<0.001
P_4-P_5	4th	438	1.691	105.8	<0.001
P_5-P_6	5th	438	2.129	105.8	<0.001
P_6-P_7	6th	435	2.564	105.1	<0.001
P_7-P_8	7th	429	2.993	103.6	<0.001
P_8-P_9	8th	422	3.415	101.9	<0.001
P_9-P_{10}	9th	421	3.836	101.7	<0.001
P_{10}-P_{11}	10th	419	4.255	101.2	>0.01
P_{11}-P_{12}	11th	419	4.674	101.2	>0.01

P_1-St interval: 96 msec.

Control interval (P_0-P_1): 414 msec.

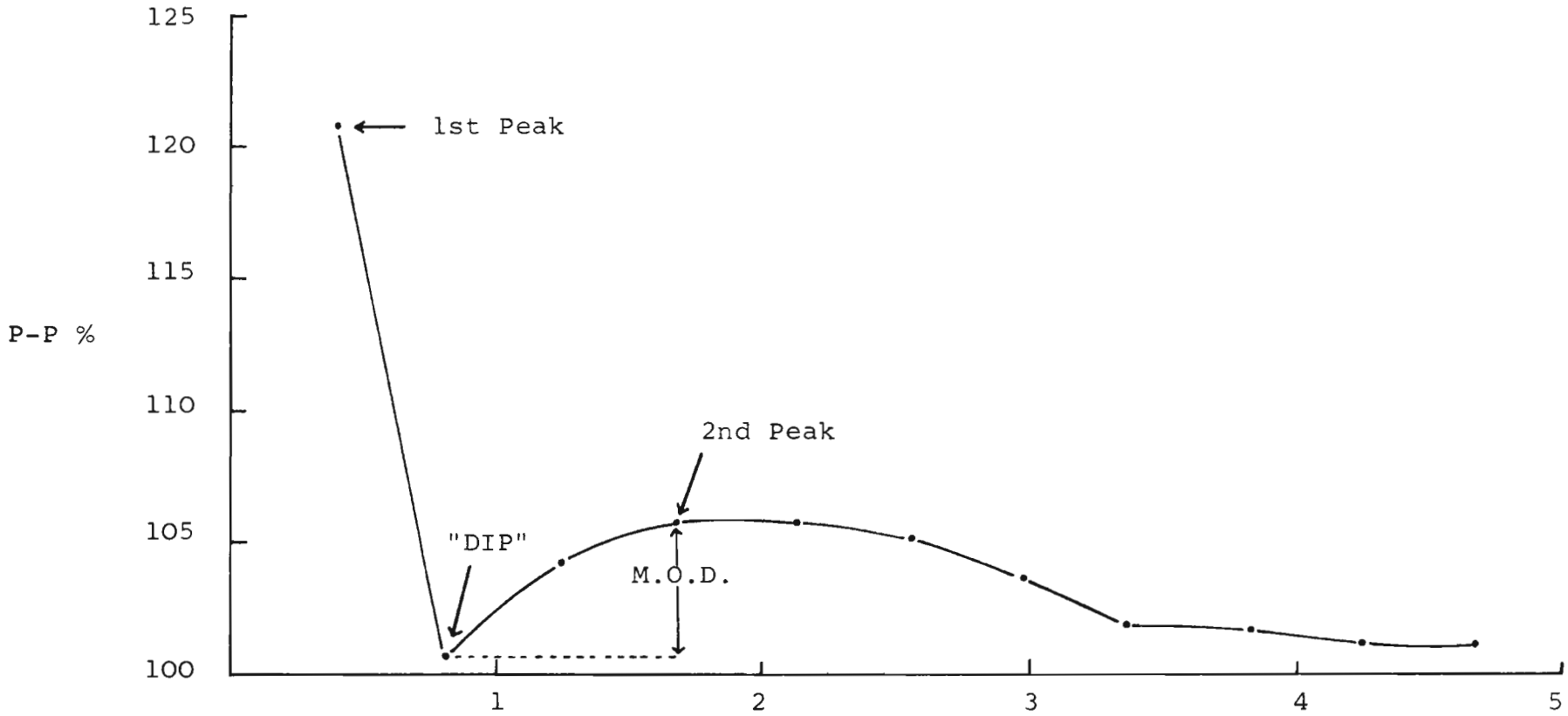


Fig. 8.

Effects of vagal stimulation on eleven cardiac cycle lengths subsequent to vagal stimulation in a single experiment. Graph plotted from data in Table III. The points represent the means of 15 responses. M.O.D. = magnitude of "dip" response.

TABLE IV

THE DURATION AND MAGNITUDE OF EFFECTS OF VAGAL STIMULATION ON CARDIAC CYCLE LENGTHS, AND THE MAGNITUDE AND TIMING OF THE "DIP" AFTER VAGAL STIMULATION

	Mean P_O-P_1 (msec)	Duration of effect (sec)	Persistence of effects in cardiac cycle	First peak (%)	P-P Interval at "dip" (%)	Second peak (%)	Magnitude of "dip" (%)	St. to "dip" time (sec)
	414	3.836	9	120.8	100.7	105.8	5.1	0.821
	497	4.727	9	131.0	108.5	110.2	1.7	1.035
	458	5.700	11	134.7	111.5	116.3	4.8	0.999
	333	1.700	5	109.6	103.9	106.8	2.9	0.642
	452	3.141	7	107.0	102.4	104.6	2.2	0.813
	712	4.254	6	113.3	100.6	104.3	3.7	1.304
	579	4.761	8	119.0	105.2	106.5	1.3	1.729
	517	2.998	6	108.5	101.2	102.2	1.0	0.888
MEAN	495	3.890		118.0	104.3	107.1	2.8	1.029
SD	114	1.255		10.4	4.0	4.4	1.6	0.343

P-P intervals at the first peak, "dip" and second peak are expressed as a percentage of P_O-P_1. Magnitude of "dip" is obtained by subtracting the P-P% interval at the "dip" from the P-P% interval at the second peak.

TABLE V

DURATION OF EFFECTS ON CARDIAC CYCLE LENGTHS WHEN THE VAGI WERE STIMULATED AT SHORT AND LONG P_1-St INTERVALS

Mean P_O-P_1	SHORT P_1-St INTERVAL			LONG P_1-St INTERVAL		
	P_1-St (msec)	Duration of effects (sec)	No. of cardiac cycles involved	P_1-St (msec)	Duration of effects (sec)	No. of cardiac cycles involved
712	215	4.254	6	574	4.499	7
581	176	4.761	8	414	4.447	8
517	197	2.998	6	429	2.722	6

TABLE VI

DURATION OF EFFECTS ON CARDIAC CYCLE LENGTHS AND THE MAGNITUDE OF THE "DIP" WHEN THE VAGI WERE STIMULATED BEFORE AND AFTER PROPRANOLOL

	Mean P_O-P_1 (msec)	Duration of effects (sec)	No. of cardiac cycles involved	Magnitude of "dip" (% of P_O-P_1)
Before Propranolol	414	3.837	9	5.1
After Propranolol	417	3.431	8	3.6
Before Propranolol	497	4.727	9	1.7
After Propranolol	469	4.967	10	1.1
Before Propranolol	458	5.700	11	4.8
After Propranolol	478	5.762	11	6.6

particular cardiac cycle subsequent to the stimulation was measured ($St-P_{2 \ldots 12}$). The durations of cardiac cycles subsequent to vagal stimulation were expressed as a percentage of the mean P_0-P_1. Finally, Student's t-test was employed to determine whether there was any significant difference at the $p = 0.01$ level between the mean durations of the cardiac cycles subsequent to vagal stimulation and the control cardiac cycle length (Table III). These results were plotted graphically (Fig. 8).

In addition, in the first three experiments the responses to vagal stimulation when the P_1-St intervals were long, were analysed in the same way.

In the two experiments where the stellate ganglia were stimulated with single supramaximal electrical shocks, the method of analysis was similar, the only difference being that the duration of only four cardiac cycles subsequent to stimulation of stellate ganglia were measured.

In the three experiments where the vagi were stimulated after administration of propranolol and atropine, the method of analysis was the same as described above.

RESULTS

In eight experiments in which the stellate ganglia were excised and the vagi were stimulated with single supramaximal shocks, the effects persisted over 3.89 sec (SD±1.255, Table IV), the number of cardiac cycles involved varying between 5 and 11. An interesting feature of these results was that the response to vagal stimulation demonstrated a "dip" (Fig. 8) i.e. after the response to vagal stimulation had reached a peak, it declined rapidly to reach a minimum and then increased again in the subsequent cardiac cycle to reach a second peak, before the response declined again. At the first peak response the cardiac cycle was lengthened to 118.0% (SD±10.4), whereas at the second peak the cycle was 107.1% (SD±4.4). At the "dip" it was 104.3%

(SD±4.0). The magnitude of the "dip" was obtained by subtracting the P-P interval at the "dip" from the P-P interval at the second peak and it was 2.8% (SD±1.6). The "dip" occurred 1.029 sec (SD±0.343) after stimulation of vagus nerves (Table IV).

The first three experiments, where the results of vagal stimulation at both short and long P_1-St intervals were analysed, showed that the duration of the effects of vagal stimulation was independent of when during the P-P interval the vagi were stimulated (Table V).

In the three experiments where the vagi were stimulated both before and after the injection of propranolol the responses to vagal stimulation were similar i.e. both showed the "dip" phenomenon and the duration of the effects were similar (Table VI).

In the two experiments where repetitive stimulation of the stellate ganglia had reduced the P-P interval there was no significant change in the cardiac cycle length when the stellate ganglia were stimulated with single supramaximal electrical shocks. Those cats given propranolol and atropine showed no significant change in cardiac cycle length in response to vagal stimulation.

DISCUSSION

The effects of vagal stimulation persisted for 3.89 sec (SD±1.255), the number of cardiac cycles involved being between 5 and 11. Levy *et al* (1970) found that in dogs the effects of vagal stimulation persisted for 10 cardiac cycles. In the present series the duration of the effects of vagal stimulation did not depend upon when during the P_1-P_2 interval the vagi were stimulated (Table V). This was not surprising as the amount of acetylcholine released by each vagal volley and the rate of destruction of the released acetylcholine by cholinesterase and hence the decay of vagal effects, would be independent of when during the P_1-P_2 interval the vagi were stimulated.

The "dip" in the responses to vagal stimulation with

single shocks was present in all 8 experiments done in the present series. Similar "dip" in responses to vagal stimulation had been obtained by Brown and Eccles (1934) in 15 out of 19 experiments done on cats, and by Levy et al (1970) in 8 out of 10 experiments on dogs. The "dip" could have been due to the stimulation of sympathetic fibres at the same time as the vagal fibres, as in fact the vago-sympathetic trunks were being stimulated in all these experiments. This possibility was discounted by showing that the stimulation of the stellate ganglia with single supramaximal electrical shocks had no effect on the P-P interval even though stimulation at a high frequency had caused a shortening of the P-P interval. Furthermore, the "dip" in the response to vagal stimulation persisted even after administering propranolol which could have blocked any sympathetic effects present. The possibility that propranolol had not blocked the sympathetic effects on the heart and that the "dip" in the response to stimulation of the vago-sympathetic trunk could still have been due to stimulation of sympathetic fibres, was ruled out by showing that after further injection of atropine, stimulation of the vago-sympathetic trunk had no effect on the P-P interval. Hence the "dip" phenomenon must be inherent in the response to the stimulation of the vagus nerves themselves.

A possible explanation of the "dip" phenomenon is as follows: the acetylcholine released from vagal terminals increases the permeability of the pacemaker cell membrane to K^+ and hence K^+ diffuses out of the pacemaker cells down an electrochemical gradient. It is postulated that K^+ diffusing out of the cell accumulates in the interstitial fluid surrounding the pacemaker cells, including the T-tubules, causing a momentary increase in the concentration of K^+ in the interstitial fluid ($[K^+]_o$). It has been shown that an increase in $[K^+]_o$ causes a shortening of the P-P interval by making the diastolic potential of the pacemaker cells less negative, even though the slope of diastolic depolarization is actually decreased (Lu, 1970).

Therefore the nett effect on the P-P interval after vagal stimulation would be dependent upon the interaction of cholinergic effects, which would cause prolongation of the P-P interval and of raised $[K^+]_o$ following on the cholinergic action, which would cause shortening of the P-P interval. The cholinergic action would be operative first, causing a prolongation of the P-P interval. Then the rising concentration of K^+ in the interstitial fluid surrounding the pacemaker cells together with the falling concentration of acetylcholine due to the action of cholinesterase would rapidly shorten the P-P interval. However, the increase in $[K^+]$ in the interstitial fluid surrounding the pacemaker cells is momentary, and therefore its effect would decline rapidly, whereas the cholinergic effects persist for a longer period. The unopposed cholinergic effect would cause the subsequent prolongation of the P-P interval and a second peak. The reduction in the P-P interval after the second peak would be due solely to acetylcholine hydrolysis by cholinesterase.

SUMMARY

The effects on the cardiac cycle length of stimulating the vagus nerves with single supramaximal electrical shocks depended upon when they were stimulated during the cycle. A maximum prolongation of the cardiac cycle was obtained when the vagi were stimulated 167 msec (SD$\pm$64) after the peak of an electrocardiogram P wave. The interval between a P wave and the subsequent vagal stimulation was called P_1-St interval. $P_1\text{-St}_{(max)}$ was the P_1-St interval at which maximum prolongation of the cardiac cycle occurred. $P_1\text{-St}_{(max)}$ increased significantly ($p < 0.001$) with longer cardiac cycles. When the P_1-St intervals were shorter or longer than 167 msec (SD$\pm$64) the effects of vagal stimulation were less. The latent period for the effects of vagal stimulation was 195 msec (SD$\pm$32). The latent period also increased significantly ($p < 0.01$) with longer cardiac cycles.

The rise time of the vagal effect, obtained by subtracting (P_1-$St_{(max)}$+ latent period) from the control cardiac cycle length, was 124 msec (SD±31) and occurred between P_1-St intervals of 167 msec (SD±64) and 291 msec (SD±70). The rise time did not vary with cardiac cycle length ($p > 0.1$), but the magnitude of the maximum response to vagal stimulation was inversely proportional to rise time ($p < 0.02$). The peak response to vagal stimulation must have occurred when the vagal effects began somewhere in the middle of diastolic depolarization of the pacemaker cells in the S-A node. The reasons for this were discussed. The half-decay time for the effects of vagal stimulation was 210 msec (SD±102).

The slope of the curve relating the prolongation of the cardiac cycle length to P_1-St is positive at P_1-St intervals less than 167 msec (SD±64) and negative at P_1-St intervals between 167 msec (SD±64) and 291 msec (SD±90). The positive slope ranged from 0.13 to 0.48 with a mean of 0.23.

The paradoxical responses of the S-A node to vagal inhibitory input obtained by Reid (1969), Levy et al (1969) and Dong and Reitz (1970) would be explained by the dependence of the cardiac cycle length upon the time of arrival of vagal stimulus in relation to the previous P wave and upon the slope of the curve relating the prolongation of the cardiac cycle length to P_1-St interval being positive and between zero and two at P_1-St intervals less than 167 msec (SD±64).

The effects of single shock stimulation of the vagus nerves persisted for 3.890 sec (SD±1.255); the number of cardiac cycles involved varied between 5 and 11. The duration of the effects of vagal stimulation did not depend upon when during the cardiac cycle the vagi were stimulated. A "dip" in the response to vagal stimulation was present in all the experiments. The possibility of the "dip" phenomenon being due to simultaneous stimulation of the sympathetic fibres in the vago-sympathetic

trunk was ruled out. It is suggested that the "dip" phenomenon may be due to transient accumulation of K^+ in the interstitial fluid surrounding the pacemaker cells in the S-A node.

CHAPTER III

RESPONSES OF MUSCULATURE OF THE RABBIT DISTAL COLON TO SYMPATHETIC NERVE STIMULATION

1. RESPONSES OF THE RABBIT DISTAL COLON TO INCREASING FREQUENCY OF SYMPATHETIC NERVE STIMULATION RATE

EXPERIMENTAL METHOD

13 Adult rabbits were used. The distal colon together with its sympathetic nerve supply was dissected from freshly killed rabbits according to the technique described by Garry and Gillespie (1954). The only modification was that the pelvic nerves were not dissected. The preparation was set up in an isolated organ bath, bathed in a physiological saline (NaCl 0.78% w/v, KCl 0.035% w/v, $NaHCO_3$ 0.137% w/v, $NaH_2PO_4 \cdot 2H_2O$ 0.0165% w/v, $CaCl_2 \cdot 2H_2O$ 0.028% w/v, $MgCl_2 \cdot 6H_2O$ 0.001% w/v, glucose 0.14% w/v, pH = 7·4) at 37°C and aerated with 95% O_2 and 5% CO_2. The movements of the gut were recorded either on a smoked drum with a frontal writing isotonic lever or on a Beckman Type RM dynograph recorder using an isotonic transducer (Harvard Apparatus heart/smooth muscle transducer No. 386). The arrival of stimuli were recorded simultaneously on a second channel. Fluid electrodes (Garry and Wishart, 1951) were used to stimulate the sympathetic nerves with supramaximal shocks (15 to 30 V and a pulse duration of 2 to 3 msec).

In 8 preparations the sympathetic nerves to the colon were stimulated at a certain frequency for 1 to 2 minutes; the intervals between the shocks were regular. Once the preparation had recovered the sympathetic nerves were stimulated for 30 seconds at the same frequency as previously and then the frequency was continuously increased, so that the intervals between the shocks were progressively decreased while the gut movements were recorded.

As maximum inhibition of the rabbit distal colon is obtained when the sympathetic nerves are stimulated at 100 pulses per sec (Garry and Gillespie, 1955) it was decided to investigate the effects of regularly repeated bursts of high frequency stimuli.

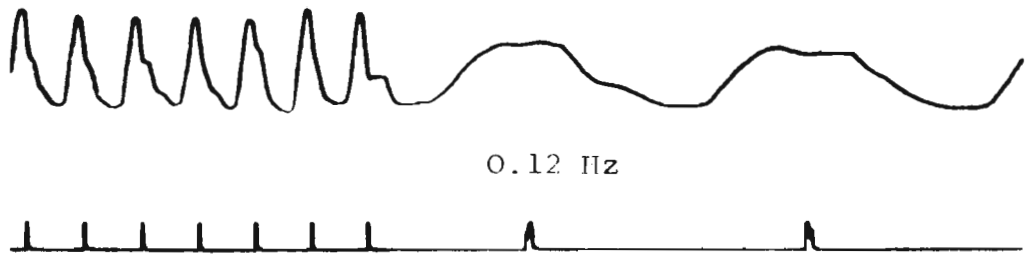
In the other 5 preparations the sympathetic nerves were therefore stimulated with bursts of stimuli, each burst consisting of 5 to 40 impulses, 10 msec apart. In any one experiment the number of impulses per burst was kept constant but the intervals between bursts were varied. The burst repetition frequency was lower than the intrinsic frequency of contraction of the gut and ranged from 0.1 Hz to 0.2 Hz.

In one experiment the sympathetic nerves were stimulated with single bursts of stimuli manually triggered at different times during the spontaneous contraction-relaxation cycle of the gut.

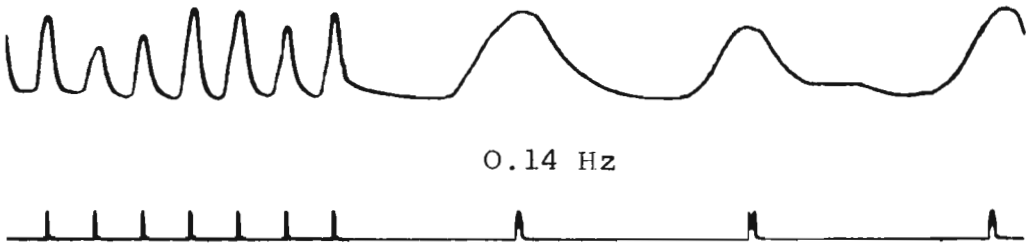
RESULTS

When the sympathetic nerves were stimulated with regular pulses at a certain frequency the magnitude and the frequency of the spontaneous contractions of the smooth muscle decreased. As the frequency of stimulation was continuously increased the magnitude of the spontaneous contraction of the smooth muscle decreased; the effects on the frequency of contraction were variable. There was usually a further decrease in contraction frequency. In a few experiments there was no change in frequency or there was an increase.

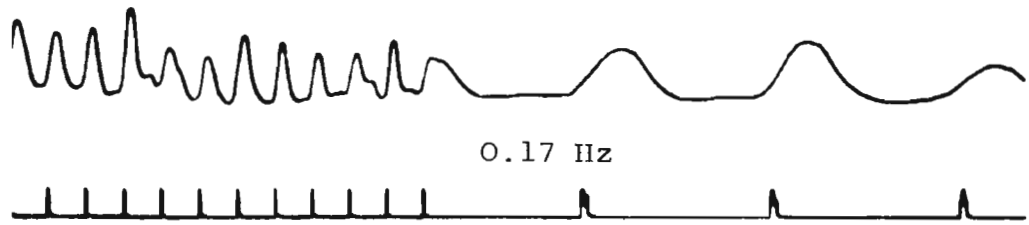
When the sympaethtic nerves were stimulated with bursts of stimuli, in 4 out of 5 preparations a 1:1 ratio existed between the frequency of bursts and the frequency of contraction. This ratio was maintained when the burst repetition frequency was increased. In one preparation a 1:1 ratio between the burst repetition frequency and the contraction frequency was obtained at the following frequencies: 0.2 Hz, 0.19 Hz, 0.17 Hz, 0.15 Hz, 0.14 Hz, 0.13 Hz and 0.12 Hz. The bursts of stimuli fell at different times during the contraction cycle of the muscle as the burst frequency was altered. At low frequencies the bursts fell during the relaxation phase and as the burst frequency was



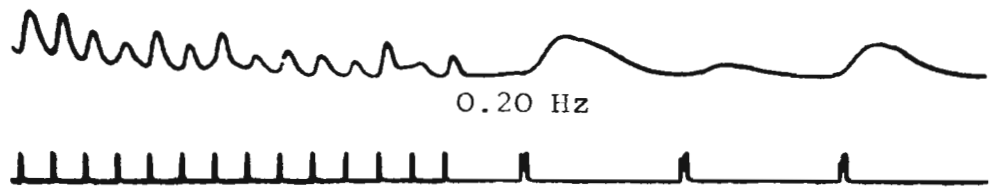
0.12 Hz



0.14 Hz



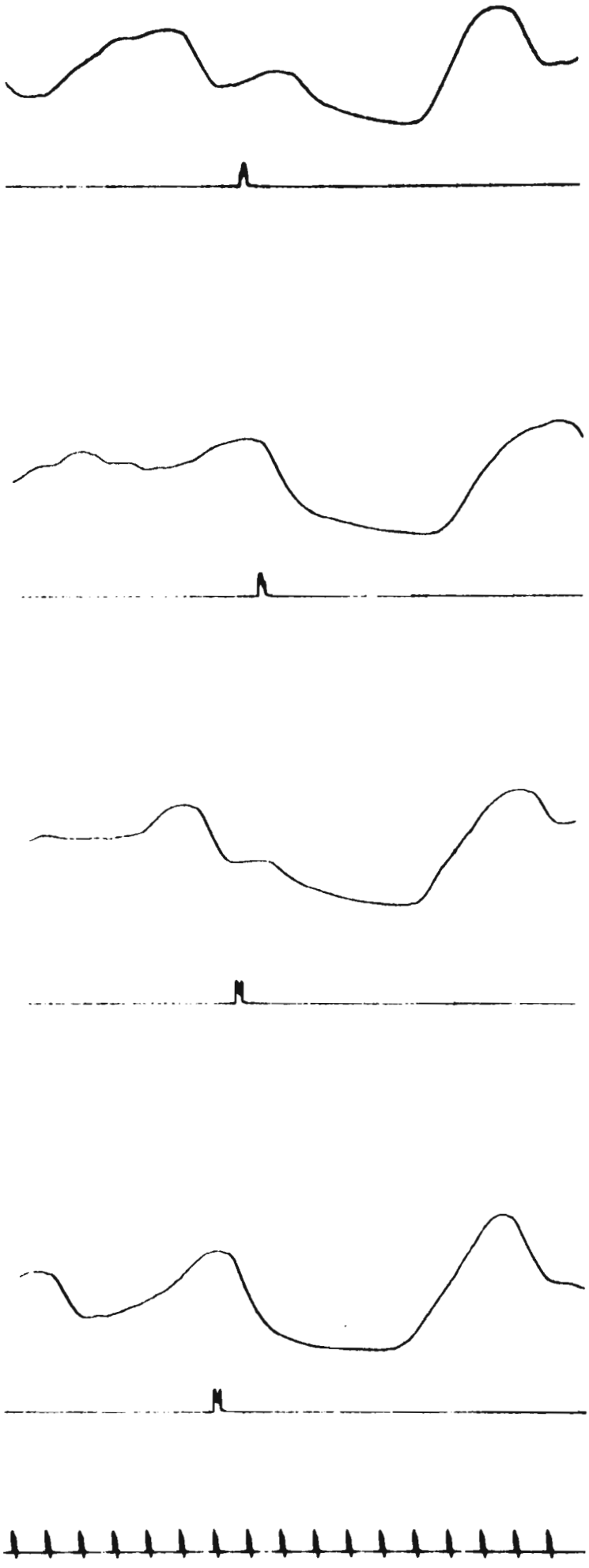
0.17 Hz



0.20 Hz

30 sec

6 sec



increased the bursts fell earlier and earlier until at the highest frequencies when there was still a 1:1 ratio the bursts fell at or slightly before the beginning of contraction (Fig. 9). The size of both tonic and rhythmic contractions decreased with increasing burst frequency. To ensure that the above responses were due to nerve stimulation and not to direct current spread to the muscle, the nerves were crushed between the electrodes and the gut; when this was done there was no response to nerve stimulation.

Increasing the frequency of bursts of stimuli applied to sympathetic nerve results in an increase in the frequency of smooth muscle contraction but a further decrease in the size of both tonic and rhythmic contraction.

When the sympathetic nerves were stimulated with single bursts of stimuli applied at different times during the contraction cycle the delay in start of the next spontaneous contraction varied (Fig. 10). The delay was maximal when the bursts were applied during the relaxation phase of the cycle and it decreased as the bursts of stimuli arrived earlier and earlier during the cycle; the delay was minimal when the bursts were applied at the beginning of the contraction. On the other hand the inhibitory effect on the size of contraction did not vary when the sympathetic nerves were stimulated with bursts of stimuli at different times during the contraction cycle.

DISCUSSION

The basis for the rhythmic mechanical contraction of the smooth muscle of the rabbit distal colon is the cyclical changes in the membrane potential of the smooth muscle cells. When the depolarization phase of the slow wave reaches the threshold potential, muscle action potentials arise and contraction follows; when the muscle cells repolarize, muscle action potentials cease and the muscle relaxes. The higher the number and frequency of muscle action potentials during a single slow wave the greater

the magnitude of contraction. Sympathetic nerve stimulation reduces the number and frequency of muscle action potential during a single slow wave and also the recurrence frequency of the slow waves and hence is inhibitory (Gillespie, 1962). A paradoxical response would be one where increasing the frequency of sympathetic nerve stimulation caused an increase in the number and the frequency of muscle action potentials and an increase in the frequency of the slow waves; this should result in an increase in the size and frequency of smooth muscle contraction. Thus by observing the size and frequency of muscle contraction one could detect the presence or absence of paradoxical responses to increasing sympathetic inhibitory input.

When the sympathetic nerves were stimulated with a regular frequency of impulses and then the frequency was continuously increased there was no paradoxical response as far as the size of the contraction was concerned. The effects on the frequency of contraction were variable. This would imply that the inhibitory effects of sympathetic nerve stimulation were not dependent upon the time of arrival of inhibitory input during the contraction cycle of the muscle. However there were more than 6 impulses regularly spaced throughout a single contraction cycle. The lowest frequency of sympathetic nerve stimulation was 2 pulses per second and the duration of contraction cycle was 3-5 sec. So even if the inhibitory effect is dependent upon the arrival time of inhibitory input during the contraction cycle, as there were many inhibitory impulses regularly spaced throughout the cycle a paradoxical response may not be seen when the frequency of inhibitory input is increased. Also, single shock stimulation has no effect on the electrical and mechanical activity of the smooth muscle cells (Gillespie, 1962).

Therefore in 5 preparations the sympathetic nerves were stimulated with bursts of stimuli and the burst repetition frequency varied. In 4 out of 5 preparations the frequency

of contraction increased as the burst repetition frequency was increased, but the size of the tonic and rhythmic contraction decreased. Thus there is a paradoxical response in the frequency but not in the size of smooth muscle contraction, to bursts of sympathetic inhibitory input.

The presence of paradoxical responses to sympathetic inhibitory input as far as the frequency of contraction is concerned leads one to conclude that the delay of the next spontaneous contraction of the smooth muscle is dependent upon the arrival time of bursts of impulses during the contraction cycle. The changes in the time relations between the bursts of stimuli and the phase of contraction at different burst repetition frequencies support this conclusion. As the burst repetition frequency was increased the bursts of stimuli fell earlier and earlier during the contraction cycle; therefore it would appear that the stimulation was least effective when the burst of stimuli arrived early during the contraction cycle and most effective when the burst arrived later. This was shown to be so when the sympathetic nerves were stimulated with single bursts of stimuli at different times during the contraction cycle (Fig. 10). This is analogous to the effects of vagal stimulation on the cat S-A node, the difference being that the vagus nerves were stimulated with single shocks whereas the sympathetic nerves were stimulated with bursts of stimuli.

On the other hand the absence of paradoxical response in the size of contraction implies that the inhibitory effect of sympathetic nerve stimulation on the size of muscle contraction is independent of arrival time of bursts of stimuli during the contraction cycle. Stimulation of sympathetic nerves with single bursts of stimuli at different times during the contraction cycle demonstrated this.

On the basis of the mechanical response one may speculate on the electrical response of smooth muscle cells to

different arrival times during the slow waves of bursts of sympathetic inhibitory impulses. It seems likely that the duration of the slow waves is affected by different arrival times of bursts of stimuli, but the size of the slow wave and the number and frequency of muscle action potentials is not affected. This can be verified by recording the membrane potential of smooth muscle cell while stimulating the sympathetic nerves with bursts of impulses applied at different times during the slow wave.

Does the type of paradoxical response of the smooth muscle to sympathetic inhibitory input, as demonstrated in isolated preparation, occur under physiological conditions? For paradoxical responses to occur the natural sympathetic efferent discharge would have to occur in regular bursts, each burst consisting of impulses at a high frequency. Though the sympathetic efferent discharges to the distal colon have not and probably cannot be recorded under physiological conditions, it appears unlikely that the natural sympathetic efferent discharges would be of such a pattern. Therefore paradoxical response of the smooth muscle to sympathetic inhibitory input probably does not occur under physiological conditions.

2. RESPONSES OF THE DISTAL COLON OF THE NEWBORN RABBIT TO SYMPATHETIC NERVE STIMULATION

EXPERIMENTAL METHOD

Ten newborn rabbits, varying in age from 1 to 9 days were used. The experimental method was similar to that described earlier in this chapter. The distal colon together with its sympathetic nerve supply was set up in an isolated organ bath. The length of the distal colon was about 1.5 cms and the magnification of the isotonic lever about 8. The sympathetic nerves to the distal colon were stimulated with supramaximal stimuli (15 V and 3 msec) at 3, 5, 10 and 25 pulses per second (PPS).

RESULTS

Table VII summarises the results. Figure 11 shows the result in a day old rabbit. In all the preparations there was inhibition of both the frequency and size of smooth muscle contraction when the sympathetic nerves were stimulated at 10 or 25 PPS. In 9 out of 10 preparations inhibition was seen when the sympathetic nerves were stimulated at 5 PPS and in 3 preparations inhibition was obtained at 3 PPS. In none of the preparations was a motor response to sympathetic nerve stimulation obtained, even at low frequencies of stimulation.

DISCUSSION

The results of the present series of experiments show that the sympathetic outflow to the distal colon is inhibitory in the newborn rabbit as it is in the adult rabbit. Assuming that the cholinergic fibres would be excitatory there was therefore no evidence for the sympathetic fibres being cholinergic.

Burn (1968) reported positive motor responses of the small intestinal smooth muscle to sympathetic nerve stimulation in the newborn rabbit, particularly at low frequencies of stimulation. However he had stimulated the perivascular nerves close to the small intestine and it is possible that he was stimulating the parasympathetic and sympathetic fibres at the same time when he obtained the motor response; on the other hand in the preparation used in the present series of experiments the sympathetic nerve which was stimulated has been shown to lack any significant number of parasympathetic fibres (Boyd et al, 1962). Furthermore, Burn (1968) also reported that in a single preparation stimulation of the perivascular nerves can produce a motor response at low frequencies of stimulation (3 and 5 PPS) and inhibition at higher frequencies (10 and 20 PPS). Garry and Gillespie (1955) have shown that it is possible to get this sort of response when the parasympathetic and sympathetic fibres to the rabbit distal colon are stimulated simultaneously; thus when the parasympathetic

TABLE VII

EFFECT OF SYMPATHETIC NERVE STIMULATION OF THE SPONTANEOUS MOVEMENTS OF THE DISTAL COLON OF THE NEWBORN RABBIT

Age of Rabbit (in days)	Frequencies of sympathetic nerve stimulation 3 PPS	5 PPS	10 PPS	25 PPS
1	NE	I	I	I
1	NE	I	I	I
2	NE	I	I	I
2	I	I	I	I
3	NE	I	I	I
3	I	I	I	I
3	NE	I	I	I
6	NE	NE	I	I
9	I	I	I	I
9	NE	I	I	I

PPS = Pulses per second. NE = No effect. I = Inhibition

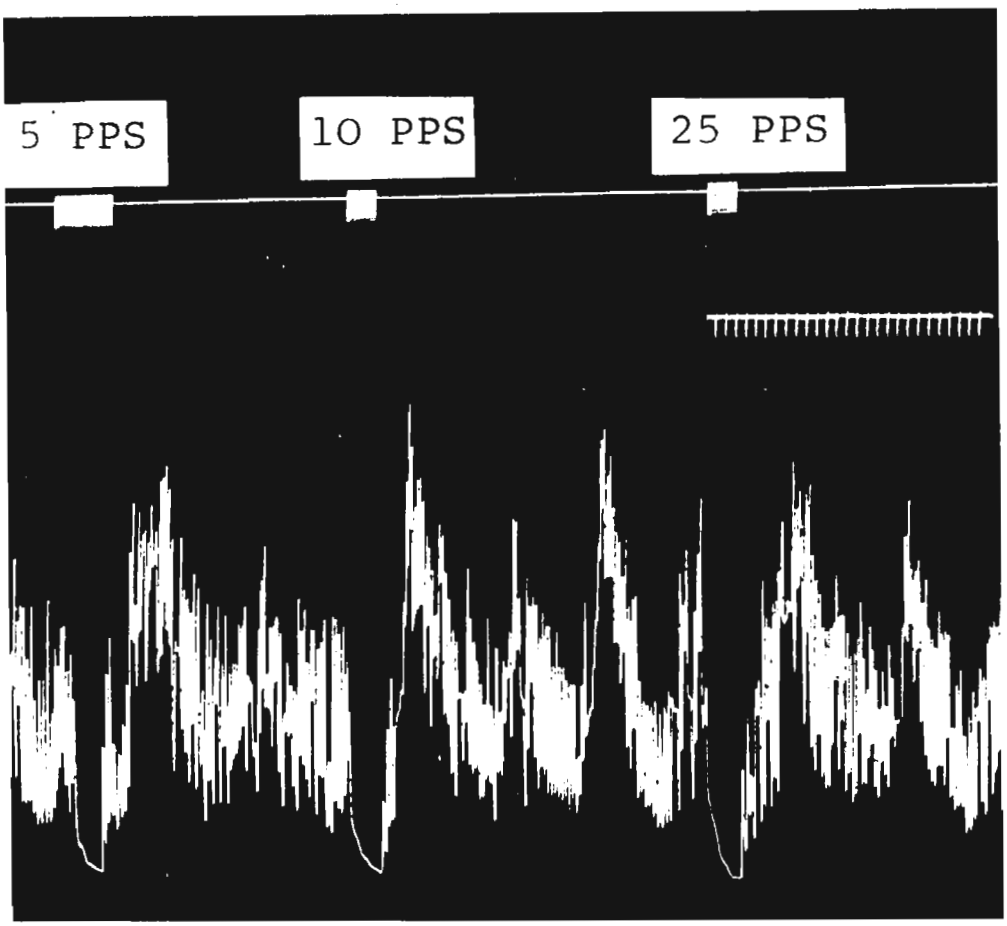


Fig. 11. Responses of isolated distal colon from a day old rabbit. Sympathetic nerves stimulated at frequencies indicated. PPS = pulses per sec. Time signal - 10 sec intervals.

and sympathetic fibres are stimulated simultaneously one can get a motor response ONLY at low frequencies of stimulation, a mixed response at intermediate frequencies and inhibition ONLY at high frequencies.

However the possibility exists that there is a phase in the development of the sympathetic fibre to the rabbit distal colon during which it is motor and cholinergic, but this is only in utero and hence cannot be demonstrated in the newborn.

SUMMARY

There was no paradoxical response of the smooth muscle in the distal colon of the adult rabbit when the frequency of sympathetic inhibitory input was continuously increased. A paradoxical response in the frequency but not in the size of the contraction of the smooth muscle was obtained when the sympathetic nerves were stimulated with bursts of stimuli, each burst consisting of 5-40 impulses, 10 msec apart. One may conclude from this that the delay of the next spontaneous contraction but not the inhibition of the size of smooth muscle contraction is dependent upon the arrival time of a burst of stimuli during a contraction cycle. This was confirmed in an experiment when the sympathetic nerves were stimulated with single bursts of stimuli applied at different times during the contraction cycle.

It is unlikely that such a paradoxical response would occur under physiological conditions as this would require the natural sympathetic efferent discharges to the smooth muscle to occur in regular bursts, each burst consisting of impulses at a high frequency.

Stimulation of the sympathetic nerves at 3, 5, 10 and 25 PPS caused an inhibition of the size and frequency of smooth muscle contraction in the distal colon of the newborn rabbit. Assuming that the cholinergic fibres are excitatory there is therefore no evidence for the sympathetic fibres to the distal

colon being cholinergic in the newborn rabbit. This is contrary to Burn's (1968) report of the sympathetic fibres being motor and cholinergic to the small intestinal smooth muscle in the newborn rabbit.

CHAPTER IV

THE HUMAN HEART RATE AND MINUTE VENTILATION IN EXERCISE AND POST-EXERCISE PERIOD

EXPERIMENTAL METHOD

The subjects were 14 men whose ages varied from 21 to 32 years. The heart rate, volume of expired gas and ventilatory chest excursions of the subjects were recorded on a 4-channel Beckman Type RM dynograph recorder.

The instantaneous heart rate was obtained by feeding an electrocardiogram signal into a Beckman Type 9857B cardiotachometer coupler. The electrocardiogram was obtained from two electrodes, one on either side of the chest in the midclavicular line in the fourth or fifth intercostal space. The chest movements during ventilation were recorded by measuring chest circumference with a strain gauge pneumograph. The expired gas was collected continuously in a very low resistance Tissot spirometer. The mechanical displacement of the Tissot spirometer was converted into an electrical signal which was recorded on the Beckman recorder. Hence a record of volume of gas expired against time was obtained and the slope of this curve gave minute ventilation.

These three parameters were recorded continuously before, during and after exercising on a bicycle ergometer. The duration of the exercise was usually 20 seconds, in some cases 30 seconds. The exercise levels were 480 (mild exercise) and 600 (moderate exercise) kilogram-metre/min, pedalling at 40 and 50 r.p.m. respectively. After completion of the experiment, the subjects were given an oral dose of 100 mg acebutolol, a β-adrenergic blocker (Leary, 1971) and four hours later the experimental procedure was repeated. In one subject this procedure was repeated after intravenous administration of atropine, 0.04 mg/kg body weight, thus blocking the sympathetic, vagal and humoral effects on the heart rate.

RESULTS

Heart Rate. The heart rate increased rapidly at the onset of exercise and then gradually over the rest of the exercise period.

There was no significant difference in the initial increase in heart rate between the β-adrenergic blocked subjects and the non-blocked controls. However, the subsequent increase in heart rate during the exercise period was significantly reduced in the β-adrenergic blocked individuals ($p < 0.05$ at mild exercise level and $p < 0.01$ at moderate exercise level). In one subject with β-adrenergic and vagal blockade there was no change in heart rate during or after the exercise period.

The recovery of heart rates in the post-exercise period were examined in greater detail. It is in the post-exercise period that the vagal discharge to the S-A node increases, and if there is any paradoxical response to increasing vagal inhibitory input it would be expected to occur in this period.

The mode of recovery of heart rate in the post-exercise period was variable. In almost all the subjects there was initially a rapid decline in heart rate, but subsequently the heart rate returned to resting levels in one, or a combination of more than one of the following ways (Fig. 12):

a) Type I: the heart rate declined regularly;
b) Type II: heart rate fluctuated markedly in phase with ventilation - sinus arrhythmia;
c) Type III: heart rate fluctuated, but not in phase with ventilation; the heart rate decreased rapidly and then increased again slowly, the whole cycle of decrease and increase in heart rate taking about 6 sec;
d) Type IV: sudden decrease in heart rate;
e) Type V: an increase in heart rate of 6 beats or more per minute with or without superimposed sinus arrhythmia.

Of particular interest to the present study was the Type V pattern of heart rate change. Table VIII shows the frequency of this pattern in the post-exercise period.

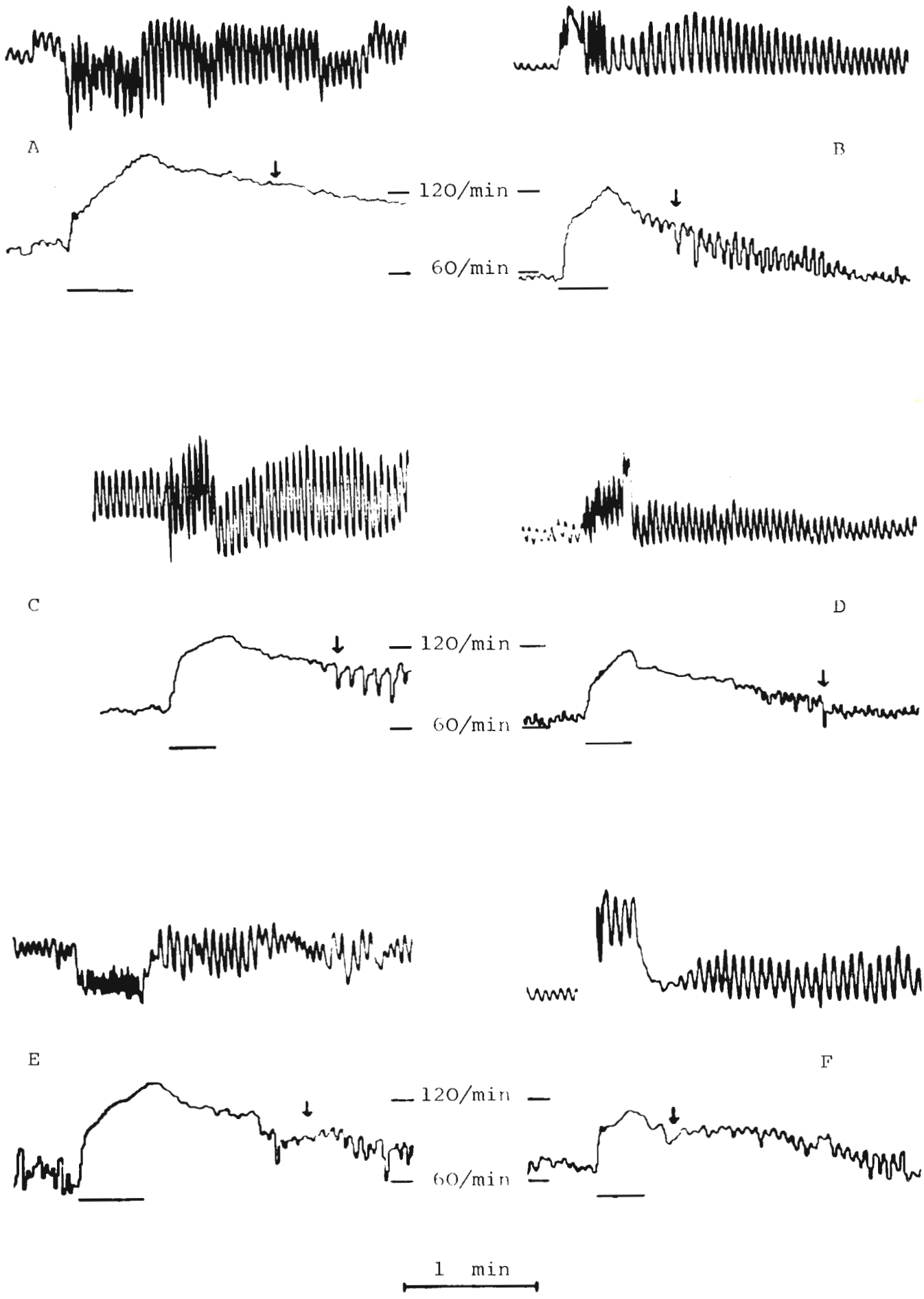

A
B
120/min
60/min
C
D
120/min
60/min
E
F
120/min
60/min
1 min

TABLE VIII

INCIDENCE OF TYPE V PATTERN OF HEART RATE CHANGE IN THE POST-EXERCISE PERIOD

	LEVEL OF EXERCISE		
	Mild	Moderate	Total
Control	$^{4}/_{14}$	$^{1}/_{14}$	$^{5}/_{28}$
β-blockade	$^{8}/_{14}$	$^{3}/_{14}$	$^{11}/_{28}$
TOTAL	$^{12}/_{28}$	$^{4}/_{28}$	$^{16}/_{56}$

NUMERATOR = number of times Type V change occurred

DENOMINATOR = number of post-exercise periods examined for Type V change

Ventilation. At the onset of exercise, minute ventilation usually increased suddenly at both levels of exercise. In the post-exercise period the changes in minute ventilation were variable. The minute ventilation returned to resting levels in one or a combination of more than one of the following ways (Fig. 13):-

a) regular slow decrease in minute ventilation;
b) sudden decrease in minute ventilation;
c) regular slow increase in minute ventilation;
d) sudden increase in minute ventilation.

The sudden increases and decreases in ventilation were usually due to changes in tidal volume, though sometimes they were due to changes in frequency of ventilation.

DISCUSSION

Heart Rate. The heart rate changes during and after exercise were due to changes in neural activity and perhaps catecholamine secretion as in one subject with β-adrenergic and vagal blockade there was no change in heart rate during or after exercise. The heart rate changes in the β-adrenergic blocked individuals would be due to changes in vagal discharge rate. Assuming that the cardiac vagal efferent discharge increases steadily in the post-exercise period in the β-adrenergic blocked individuals, the Type V pattern of heart rate change may be the equivalent of the paradoxical responses demonstrated in cat and rat (Reid, 1969) and dog (Levy et al, 1969 and Dong and Reitz, 1970) i.e. an increase in the heart rate with increasing vagal stimulation frequency. In the β-adrenergic blocked individuals the Type V pattern occurred in 11 out of 28 post-exercise periods. Consideration of the variation in the naturally occurring cardiac vagal efferent discharges may offer an explanation for the observed irregularity of occurrence of the Type V pattern.

The vagal efferent discharge to the heart in the dog (Jewett, 1964, and Katona et al, 1970) and cat (Kunze, 1972) is modulated by ventilation and arterial pressure pulse. The

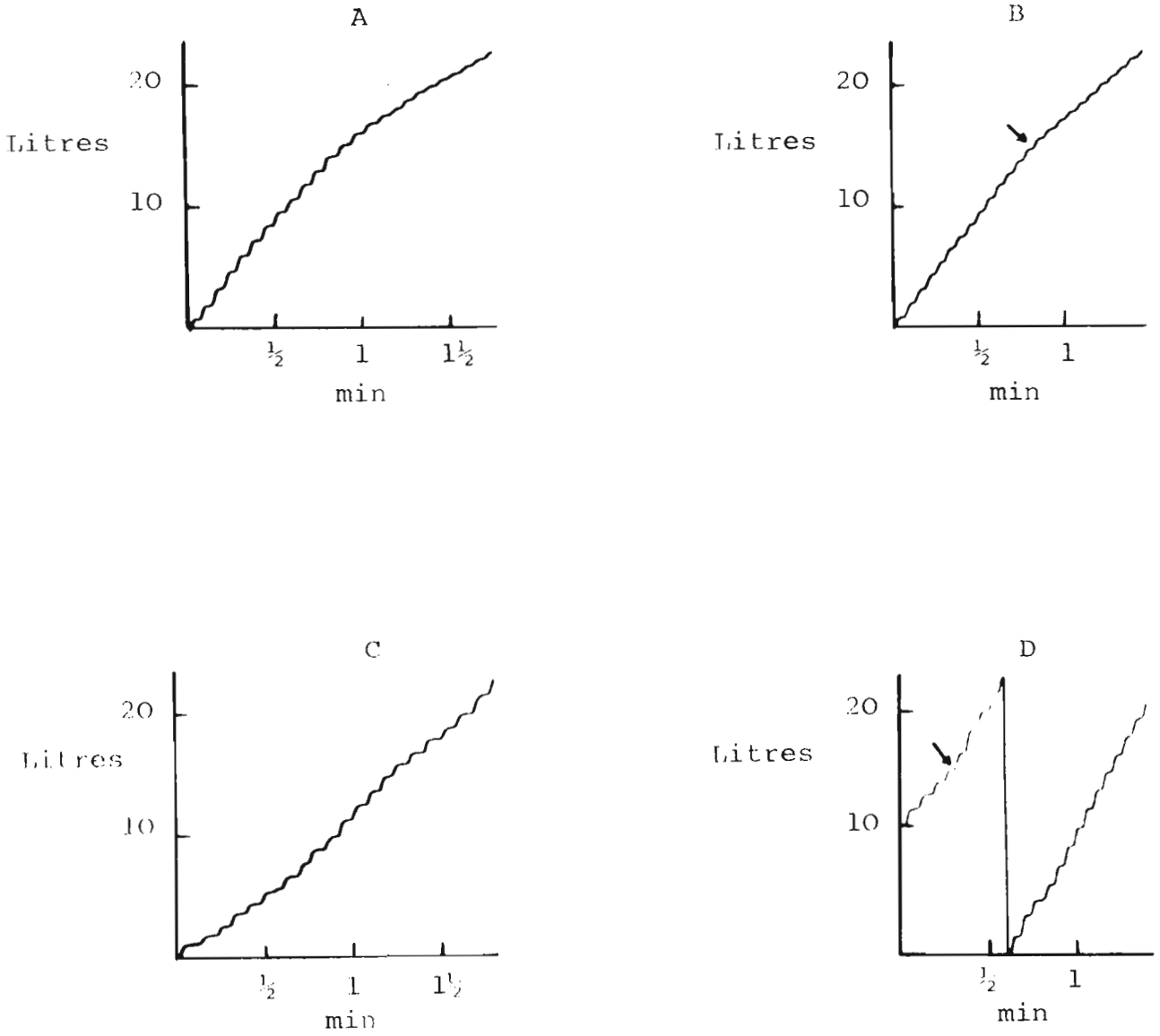


Fig. 13. Excerpts of records of volume of expired gas in the post-exercise period. Minute ventilation is given by the slope of the curve. Y-axis - volume of expired gas in litres. A, B, C and D show regular decrease, sudden decrease, regular increase and sudden increase in minute ventilation. The arrows in B and D indicate the times of sudden changes in minute ventilation. The rapid fall in D is due to electrically returning the pen to starting position.

vagal discharge is reduced or abolished during inspiration and has been shown to be time-linked to the beginning of the systolic pressure rise. The interval between the beginning of aortic pressure rise and the first impulses in the vagal discharge varied between 55 and 140 msec (Katona et al, 1970). Katona et al (1970) attributed the pulse modulation to the burst of activity of baroreceptors during the rise of systolic blood pressure. The variation in the time intervals between the systolic pressure rise and the first impulses in the vagal discharge, however, may be due to other neuronal inputs to the vagal nucleus since there was little random variation in the firing of baroreceptors from one cardiac cycle to the next (Katona et al, 1968). It is probable that the time interval between the beginning of systolic pressure rise and cardiac vagal efferent discharge would be short if the vagal neurons were facilitated and long if they were inhibited.

The grouping of the efferent vagal impulses during the cardiac cycle may prevent the physiological occurrence of paradoxical responses, since one of the criteria for a paradoxical response is that the inhibitory impulses should be regularly spaced (Perkel et al, 1964). However, Dong and Reitz (1970) have demonstrated that a paradoxical response can be obtained in the dog when the vagus nerves are stimulated with bursts of stimuli (5 impulses spaced 10 msec apart in each burst) and the frequency of bursts increased from 1.11 to 1.67 Hz. The mere grouping of vagal impulses in the cardiac cycle apparently does not prevent the physiological occurrence of paradoxical response.

The P-R interval in man at rest varies between 120 and 200 msec (Ganong, 1971) and the interval between R wave and the beginning of systolic pressure rise varies between 52-103 msec (Kroeker and Wood, 1955). Therefore the interval between the beginning of P wave and the beginning of systolic pressure rise would be between 172 and 303 msec. The interval between systolic

pressure rise and the first impulses in cardiac vagal efferent discharge in the dog was found to vary between 55 and 140 msec. If one uses this value for man, the vagal discharge would be expected to begin between 227 msec and 443 msec after the beginning of P wave. In cat the average rise time of vagal effect is 124 msec and is between 167 msec and 291 msec after the peak of P wave (see page 44) or between 190 msec and 314 msec after the beginning of P wave. There are no figures available for rise time in man but it is possible that rise time for vagal effects is between 227 and 443 msec after the beginning of P wave. If this estimate is correct, it is not surprising that paradoxical responses do not occur frequently because for paradoxical responses to occur the vagal discharge must not occur during the rise time. If the discharge occurs and its timing varies within the rise time then the orthodox response would be obtained i.e. further slowing of the S-A rate with an increase in vagal inhibitory discharge.

It is probable that in man pulse modulation of the cardiac vagal efferent discharge normally serves to confine the variation in timing of the discharge to the rise time and thereby prevent the occurrence of paradoxical response. However the cardiac vagal efferent discharge may occur at times other than during the rise time; paradoxical responses would then be obtained with increasing vagal inhibitory discharge.

In the β-adrenergic blocked individuals the Type V pattern of heart rate (and hence a probable paradoxical response) occurred in 11 out of 28 post-exercise periods, whereas in non-β adrenergic-blocked controls it occurred in only 5 of 28 instances. It would appear that intact and functioning sympathetic supply to the heart reduces the occurrence of probable paradoxical responses in the post-exercise period. The sympathetics may act by decreasing the P-R interval (Ganong, 1971) so that the cardiac vagal efferent discharge would occur mainly during rise time. Another possible reason is that the variation in the sympathetic

activity would alter the S-A rate and hence mask a paradoxical response to increasing vagal input to the S-A node. This might be so if the effect of sympathetic activity on the S-A node was rapid and if the S-A node responded to single discharges of sympathetic nerves. However, the effects of sympathetic stimulation on the S-A node occur slowly, the peak response taking 10-20 sec depending on the frequency of stimulation (Warner and Cox, 1962). The node does not respond to single shock stimulation of the stellate ganglia (see page 42). It is unlikely that reduction in the occurrence of probable paradoxical response by sympathetic activity would be due to its effects on the S-A node.

The Type V pattern of heart rate change and thus a possible paradoxical response was present in only 1 out of 14 subjects at a moderate level of exercise, whereas at a mild level of exercise it was present in 4 out of 14 subjects. This difference may be due to the greater increase in sympathetic activity to the heart in moderate levels of exercise when compared with mild exercise levels.

The activity of the vagal nucleus and hence the vagal discharge is influenced by neural input from many sources. These include baroreceptors situated in large arteries and veins, atria and ventricles, chemoreceptors, pain afferents, respiratory centre, hypothalamus and cerebral cortex. As a result of this the vagal discharge may not increase steadily in the post-exercise period, but may fluctuate even though the overall trend may be one of increase. If this were so, then the Type V pattern may not be the equivalent of paradoxical response demonstrated in animals. One of the theoretical ways of resolving this would be to monitor the cardiac vagal efferent discharge and heart rate in the post-exercise period in β-adrenergic blocked individuals! Then the Type V pattern in the post-exercise period could be confirmed as a paradoxical response if the cardiac vagal efferent discharge

increased at the same time.

The Types I and II, and Types I and III patterns in the post-exercise period have been described previously by Davies and Neilson (1967) and Lamb (1963) respectively. The Type IV pattern i.e. sudden drop in heart rate, was usually associated with a sudden decrease in minute ventilation and was probably due to the influence of respiratory centres on the cardiac centres in the brain stem.

The initial increase in heart rate during exercise was not influenced by β-adrenergic blockade and therefore must have been due to decreased vagal activity. The subsequent increase in heart rate during exercise, though still present, was significantly decreased by β-adrenergic blockade and therefore must have been in part due to increased sympathetic activity. The increase in heart rate at the onset of exercise is due primarily to a decrease in the cardiac vagal efferent discharge, whereas the subsequent increase in heart rate is due to both a further decrease in vagal discharge and an increase in sympathetic discharge to the S-A node. This has been described previously (Smulyan and Eich, 1968; Epstein et al, 1965).

Ventilation. The minute ventilation in the post-exercise period increased or decreased either slowly or suddenly.

One may regard the respiratory centres in the brain stem as a "black box" with excitatory and inhibitory inputs and an output to the respiratory muscles, so that a certain minute ventilation may be achieved. In the post-exercise period the excitatory input to the respiratory centres would be withdrawn and the inhibitory input increased. If one assumed that the excitatory input to the respiratory centres would be withdrawn steadily and the inhibitory input increased steadily, then one would expect a gradual decline in minute ventilation. But this was not the only pattern of change in minute ventilation in the

post-exercise period. The minute ventilation in fact also increased, gradually or suddenly. It is possible that the increase in minute ventilation in the post-exercise period is a form of paradoxical response which occurs despite the enormous complexity of the whole system. The sudden decrease in minute ventilation may also be a form of paradoxical response since the minute ventilation would have been expected to decrease slowly and regularly with a steadily decreasing excitatory input and steadily increasing inhibitory input.

On the other hand it is possible that the inhibitory input to the respiratory neurones may not be increasing steadily nor the excitatory input declining steadily in the post-exercise period. The respiratory neurones, as pointed out previously, receive neural input from many sources and it is possible that the algebraic summation of the neural input and consequently the output of the respiratory centre may not be declining steadily in the post-exercise period. If this were so, then the increases and sudden decreases in minute ventilation in the post-exercise period would not be a form of paradoxical response. The "hunting" behaviour of the ventilatory control system may explain the increases in the minute ventilation in the post-exercise period.

SUMMARY

The heart rate increased rapidly at the onset of exercise and then more gradually over the rest of the exercise period. The initial increase in the heart rate during exercise was not affected by β-adrenergic blockade but the subsequent increase in heart rate was significantly reduced by β-adrenergic blockade. Hence the increase in heart rate at the onset of exercise is due primarily to a decrease in the cardiac vagal efferent discharge, whereas the subsequent increase in heart rate is due to both a further decrease in vagal discharge and an increase in sympathetic discharge to the S-A node.

In almost all the subjects there was initially a rapid decline in the heart rate in the post-exercise period, but subsequently the heart rate returned to resting levels in a variety of ways. These were classified into 5 types. Of particular interest to the present study was the Type V pattern of heart rate change. This was characterised by an increase in heart rate of 6 beats or more per minute during the post-exercise period, with or without superimposed arrhythmia. The Type V pattern may be the equivalent of the paradoxical responses to inhibitory input demonstrated in animal experiments i.e. an increase in the heart rate with increasing vagal stimulation frequency. Type V pattern occurred more frequently at mild exercise levels (4 out of 14) than at moderate exercise level (1 out of 14) and also more frequently in β-adrenergic blocked individuals (11 out of 28) than in control subjects (5 out of 28). It is suggested that the sympathetic effects on the P-R interval and arterial baroreceptor modulation of vagal efferent discharge protect against the occurrence of paradoxical responses to vagal inhibitory input. They may do so by confining the vagal discharge to the rise time of vagal effect during the cardiac cycle.

On the other hand the Type V pattern in β-adrenergic blocked individuals may be due to a decrease in the vagal discharge, in which case Type V pattern would not be a paradoxical response.

The changes in minute ventilation in the post-exercise period were also variable. Besides a gradual decline in minute ventilation there were also gradual increases and sudden increases and decreases in minute ventilation. These may represent a form of paradoxical response to increasing inhibitory input and decreasing excitatory input to the respiratory neurones in man. However, all the changes in minute ventilation could also be explained by fluctuating excitatory and inhibitory neural input to the respiratory neurones.

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The vagus nerves of the cat were stimulated with single supramaximal shocks at various times during the cardiac cycle. The resultant effect on the pacemaking activities of the sinoatrial (S-A) node depended upon the time of arrival of the vagal volley during the cycle. The slope of the curve relating the prolongation of the cardiac cycle length to the interval between the stimulus and the previous P wave (P_1-St) was positive and ranged between 0.13 and 0.48 at P_1-St intervals less than 167 msec ($\pm$64). These findings explain the paradoxical responses of the S-A node to vagal stimulation reported by Reid (1969), Levy et al (1969) and Dong and Reitz (1970). The peak response to vagal stimulation occurred when the vagal effects began somewhere about the middle of diastolic depolarization of the pacemaker cells in the S-A node and not at the beginning of diastolic depolarization as suggested by Levy et al (1970).

The effects of single shock stimulation of the vagus nerves on the cardiac cycle lengths persisted for 3.89 sec (SD$\pm$1.255), the number of cardiac cycles involved varying between 5 and 11. A "dip" in the response to vagal stimulation was present in all the experiments. The "dip" may be due to transient accumulation of K^+ in the interstitial fluid surrounding the pacemaker cells in the S-A node.

Varying the frequency of sympathetic nerve stimulation elicited no paradoxical response from the smooth muscle of the rabbit distal colon. However, stimulating the sympathetic nerves with bursts of stimuli produced a paradoxical response in the frequency but not in the size of smooth muscle contraction. This leads one to conclude that the delay of the next spontaneous contraction but not the inhibition of size of contraction is dependent upon the arrival time of bursts of stimuli during a contraction cycle. This was confirmed in an experiment where the sympathetic nerves were stimulated with single bursts of

stimuli applied at different times during the contraction cycle. It is unlikely that such a paradoxical response would occur under physiological conditions as this would require the natural sympathetic efferent discharges to the smooth muscle to occur in regular bursts, each burst consisting of impulses at a high frequency.

The sympathetic outflow to the smooth muscle in the distal colon of the newborn rabbit is inhibitory, as it is in the adult rabbit. This is contrary to Burn's (1968) report of the sympathetic fibres being motor and cholinergic to the small intestinal smooth muscle in the newborn rabbit.

The heart rates in post-exercise period in men returned to resting levels in a variety of ways. These were classified into 5 types. Of particular interest to the present study was the Type V pattern which was characterised by an increase in the heart rate of 6 beats or more per minute in the post-exercise period with or without superimposed sinus arrhythmia. Assuming that the cardiac vagal efferent discharge increases progressively in the post-exercise period the Type V pattern may be the equivalent of paradoxical responses to vagal inhibitory input demonstrated in animal experiments. Type V pattern of heart rate change occurred rarely in control subjects and somewhat more frequently in β-adrenergic blocked subjects. It is likely that the sympathetic effects on the P-R interval and arterial baro-receptor modulation of vagal efferent discharge protect against the occurrence of paradoxical response to vagal inhibitory input under physiological conditions. They may do so by confining the vagal discharge to the rise time of vagal effect during the cardiac cycle.

However, it is possible that vagal discharge may not increase progressively in the post-exercise period; it may also decrease in which case Type V pattern would be an orthodox response to a decrease in vagal discharge.

The minute ventilation in post-exercise period increased or decreased either slowly or suddenly. The gradual increases and sudden changes in minute ventilation may represent a form of paradoxical response to increasing inhibitory and decreasing excitatory input to respiratory neurones. However, the respiratory neurones receive neural input from many sources and it is possible that the algebraic sum of the neural input and consequently the output of the respiratory neurones may not be declining steadily in the post-exercise period. The fluctuating excitatory and inhibitory inputs to the respiratory neurones may account for the gradual increases and sudden changes in minute ventilation. It is likely that paradoxical responses of respiratory neurones do not occur because they receive excitatory and inhibitory inputs from many sources.

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