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EFFECTS OF VAGAL STIMULATION AND APPLIED ACETYLCHOLINE ON PACEMAKER POTENTIALS IN THE GUINEA-PIG HEART

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SUMMARY

1. Intracellular recordings were made from pacemaker cells lying in the sino-atrial node of guinea-pigs.

2. Low-frequency vagal stimulation slowed the rate of generation of pacemaker action potentials; high-frequency stimulation stopped the generation of action potentials.

3. During vagal stimulation the rate of diastolic depolarization was reduced with the action potential otherwise unchanged; when the heart stopped the membrane potential of pacemaker cells settled to a value positive of the maximum diastolic potential.

4. In contrast, added acetylcholine caused membrane hyperpolarization and shortened the duration of action potentials.

5. The effects of both added acetylcholine and vagally released acetylcholine were abolished by hyoscine.

6. It is suggested that neurally released acetylcholine acts to change the balance between inward and outward current flow during diastole by modifying the properties of existing voltage-dependent channels. In contrast added acetylcholine appears to activate a different set of receptors which increase the potassium conductance of pacemaker cells.

INTRODUCTION

In virtually all vertebrates, stimulation of postganglionic vagal fibres causes the release of acetylcholine which slows the heart by acting on pacemaker cells. Acetylcholine, applied directly to the heart, also causes cardiac slowing. Both of these effects are blocked by muscarinic receptor antagonists. It is generally held that in either case acetylcholine activates a muscarinic receptor which increases the potassium conductance, g_K, of pacemaker cells and so hyperpolarizes them (Noble, 1975; Löffelholz & Pappano, 1985).

In a recent series of experiments, intracellular recordings were made from pacemaker cells in the sinus venosus of the toad, *Bufo marinus*, and the effects of

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vagal stimulation compared with the effects of added acetylcholine (Bywater, Campbell, Edwards, Hirst & O'Shea, 1989). It was found that although both vagal stimulation and added acetylcholine each slowed and stopped the generation of pacemaker potentials, the associated membrane potential changes were different. Added acetylcholine caused a sustained hyperpolarization. During slowing the duration of pacemaker action potentials was reduced. In contrast during vagal slowing there was little change in the shape of pacemaker action potentials and only a slight increase in maximum diastolic potential. The effects of both added acetylcholine and vagal stimulation were prevented by a muscarinic receptor antagonist. It was suggested that vagally released acetylcholine might cause slowing by suppressing inward current flow during diastole whereas added acetylcholine increased outward current flow during much of the cardiac cycle (Bywater *et al.* 1989).

At first sight, this mechanism of vagal inhibition in the toad appears to be unusual. However, while there are many reports that acetylcholine causes an increase in g_K (Löffelholz & Pappano, 1985), there are only a few that suggest that acetylcholine released from the vagus increases g_K in pacemaker cells. For example, vagal stimulation of the frog heart has been shown to cause hyperpolarization of arrested pacemaker cells (del Castillo & Katz, 1955; Hartzell, 1979, 1980), but only occasionally in normally beating cells (Hutter & Trautwein, 1955, 1956). No such clear-cut hyperpolarizations have been detected from rabbit pacemaker cells on vagal stimulation (Toda & West, 1966; Shibata, Giles & Pollack, 1985; see, however, Spear, Kronhaus, Moore & Kline, 1979); indeed, records very similar to those made in the toad have been obtained.

The aim of this study was to compare the effects of added acetylcholine with the effects of vagal stimulation on pacemaker potentials recorded from a mammalian species. Isolated atrial preparations obtained from the guinea-pig were used. Essentially the same observations were made from guinea-pig sino-atrial node pacemaker cells during vagal stimulation as had been made from the toad pacemaker cells.

METHODS

Guinea-pigs (male or female, weight 300–350 g) were anaesthetized with sodium pentobarbitone (50 mg/kg, I.P.). In a trial series of experiments, the right vagus in the cervical region was isolated and, along with the entire thorax, was rapidly removed and transferred to a dissecting chamber containing oxygenated physiological saline (composition, mM: NaCl, 120; KCl, 5; $CaCl_2$, 2·5; $MgCl_2$, 2·0; NaH_2PO_4, 0·1; $NaHCO_3$, 25; glucose, 11; gassed with 95% O_2, 5% CO_2). Preparations comprising the right vagal nerve, the superior venae cavae and the left and right atria, with the ventricles cut away, were dissected out. With such preparations, it was found that stimulation of the right vagus alone always caused cardiac arrest. This is in accord with the idea that the right vagus provides the dominant innervation to the sino-atrial node and atria in mammals (see Higgins, Vatner & Braunwald, 1973). The preparations were loosely pinned in a 2 ml tissue bath and viewed using an inverted compound microscope. The preparations were continuously superfused at 6 ml/min with warmed (35 °C), physiological saline. When the tissues were treated with drugs, these were added to the physiological solution in the appropriate concentration.

Intracellular recordings were made from pacemaker cells of the guinea-pig sino-atrial node using high-resistance microelectrodes (100–150 MΩ when filled with a filtered 0·5 M-potassium chloride solution) pulled on a Brown Flaming microelectrode puller (Sutter Instruments, San Francisco). In a few early experiments, more conventional lower-resistance recording electrodes ($\sim$ 40 MΩ) were

used. Whilst these gave adequate recordings from atrial cells, maintained recordings (15–30 min) from pacemaker cells could only be obtained with the higher-resistance electrodes. It was also found that the amplitudes of action potentials recorded with high-resistance electrodes were routinely greater than those recorded with the lower-resistance electrodes. All membrane potential records were low-pass filtered, cut-off frequency 3 kHz, digitized and stored on disc for later analysis. The right vagus was stimulated at a point some 2 cm from the heart, before the entry of many sympathetic fibres, with 10 s trains of stimuli (stimulation voltage 7–10 V, pulse width 1·0 ms) of variable frequency (1–40 Hz).

Pacemaker cells were accessed through the atrio-ventricular aperture. They were identified on the basis of membrane potential records, in which a characteristic pre-potential led smoothly into an action potential (see Fig. 1*A*). They were found to lie on the dorsal surface of the right atrium near the entrances of the two venae cavae. In most experiments it was necessary to make recordings from a number of sites before pacemaker cells could be identified with confidence. Records which corresponded to 'driven' or 'follower' cells (Noble, 1975) were frequently obtained near the region of pacemaker cells (Fig. 2*B*). Driven cells were characterized in that the diastolic depolarization did not lead smoothly into an action potential; rather, the action potential arose abruptly. Beyond the region where recordings were made from pacemaker and driven cells recordings similar to those made in other atrial muscles were detected (Fig. 1*C*). The general impression gained was that only a small area of tissue behaved as a true pacemaker region (see Opthof, de Jonge, MacKaay, Bleeker, Masson-Pevet, Jongsma & Bouman, 1985). Hyoscine sulphate and acetylcholine chloride, both obtained from Sigma Chemicals, were used in some experiments.

RESULTS

General observations

Sino-atrial node cells showed a rhythmic discharge of action potentials at frequencies in the range 90–180/min. The action potentials were similar to those recorded from other pacemaker tissues except that the peak diastolic potential was often more negative than −60 mV (cf. Noble, 1975), with a mean peak diastolic potential of −65·4 mV (S.E.M. ± 1·0 mV; $n = 8$, each n value representing data from a separate animal). Action potentials had peak amplitudes of some 70–108 mV (mean = 96·4 ± 3·8 mV; $n = 8$) when measured from the maximum diastolic potential. Each action potential had a duration of about 200 ms, measured from the point of initiation to the peak diastolic hyperpolarization. The action potential was preceded by a slow diastolic depolarization which developed over some 140–250 ms and led smoothly into the action potential (Fig. 1*B*).

Recordings from cells around the pacemaker region showed action potentials that arose abruptly from the diastolic depolarization (Fig. 1*B*). During their peak diastolic hyperpolarization the membrane potential was more negative (mean −75·8 ± 1·0 mV; $n = 9$) and the action potentials had larger amplitudes (mean 112·6 ± 2·4 mV; $n = 9$) than those recorded from pacemaker cells. We assume that these records were obtained from 'driven' cells rather than true pacemaker cells (Noble, 1975).

Unless stated otherwise, all data reported here were obtained from cells that were characterized as pacemaker cells according to the criteria indicated in Fig. 1.

Effects of vagal stimulation on pacemaker potentials

Vagal stimulation at frequencies of 1–10 Hz slowed the rate of generation of action potentials (Fig. 2*A* and *B*; Fig. 3*A*; Fig. 6*A*). It can be seen that during vagal

slowing, the peak diastolic potential increased slightly. During trains of vagal stimuli at higher frequencies, 15–40 Hz, the spontaneous generation of action potentials stopped (Fig. 2 *C*). With the higher frequencies of stimulation, as the rate of generation of action potentials slowed before arrest, a small 0·5–6 mV (mean 3·1 ± 0·6 mV; $n = 8$) increase in the peak diastolic potential was frequently observed. Invariably when the generation of action potentials stopped, the membrane poten-

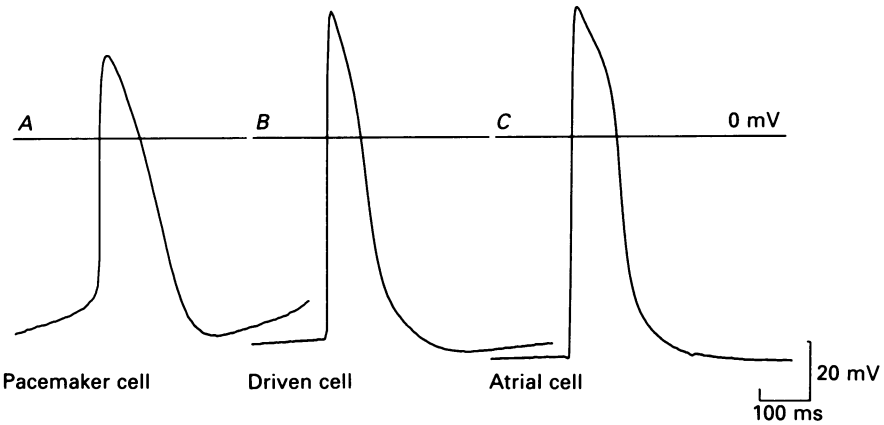

Fig. 1. Different forms of action potentials recorded in and around the sino-atrial node of the guinea-pig. A recording characterized as being made from a pacemaker cell is shown in *A*. The diastolic depolarization led smoothly into an action potential. In *B* a record made from a nearby region is shown. An abrupt inflexion is seen at the end of the diastolic depolarization; this type of recording was considered to have been made from a 'driven' or 'follower' cell. For comparison, the record shown in *C* was made from an atrial cell. Note the absence of a diastolic depolarization and the abrupt initiation of an action potential. Calibration bars apply to all records..

tial settled to a potential some 10 mV positive (range 4–15 mV; mean 10·8 ± 0·7 mV; $n = 8$) of the peak diastolic hyperpolarization. This corresponds to an absolute potential of about −55 mV. When vagal stimulation was stopped the generation of pacemaker action potentials recommenced, initially at a reduced rate. The rate returned to control values over the next 10–20 s. Immediately after the period of vagal inhibition, the peak diastolic hyperpolarization was some 1–7 mV (mean 3·4 ± 0·5 mV; $n = 8$) more negative than that recorded prior to vagal stimulation (Fig. 2*C*). The effects of vagal stimulation were prevented by adding hyoscine (1×10^{-6} M) to the perfusion fluid (Fig. 2*D*). Presumably the vagal inhibition had been mediated by the release of acetylcholine from parasympathetic postganglionic fibres.

When membrane potential records were examined using a faster display, it became apparent that the major changes in the configuration of the pacemaker potentials related to the rate of diastolic depolarization and occasionally to a changed threshold for the initiation of the action potential. These changes are illustrated in Fig. 3. The upper trace (Fig. 3*A*) shows the membrane potential change recorded at a slow scan speed; the lower part of the figure (Fig. 3*B*) shows three expansions of this trace. It can be seen that during the stimulation period the rate of action potential discharge

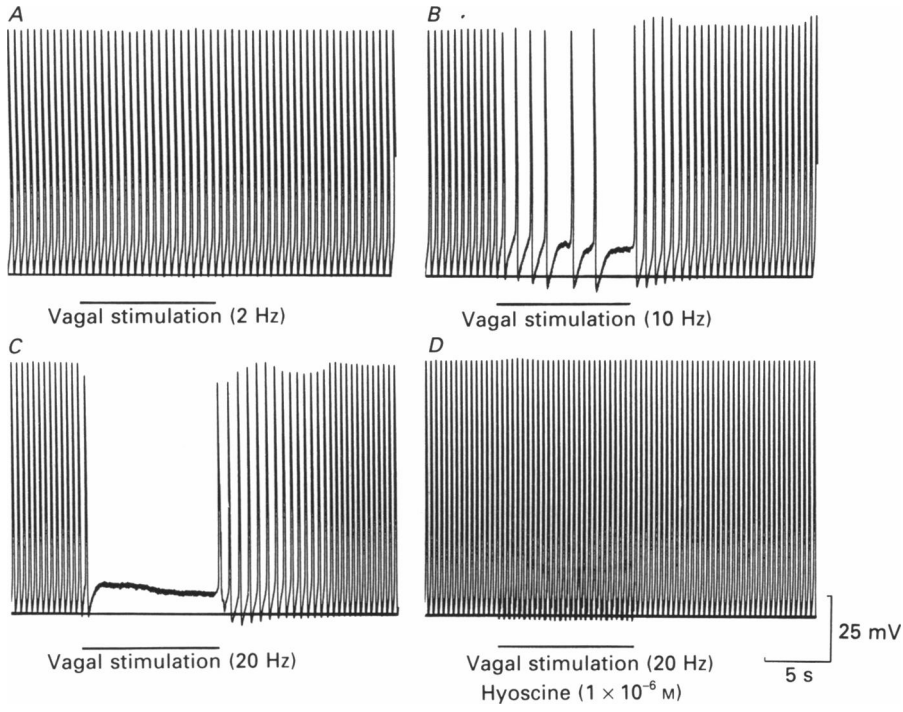

Fig. 2. Effect of vagal nerve stimulation on the membrane potential of a pacemaker cell of the guinea-pig sino-atrial node. In each record the right vagus was stimulated with supramaximal stimuli for periods of 10 s. In *A*, the stimulus frequency was 2 Hz; the heart rate fell from 120 to 110 beats/min. In *B*, the stimulus frequency was 10 Hz; the heart rate fell from 120 to about 30 beats/min. Note that during maximum slowing the peak diastolic potential only increased by some 2–4 mV. In *C*, the stimulus frequency was 20 Hz; the generation of action potentials rapidly stopped and the membrane potential settled at a value positive of the peak diastolic potential. During recovery from the period of vagal inhibition, there was a small increase (~ 7 mV) in the maximum diastolic potential. The record shown in *D* was made from the same preparation 15 min after adding hyoscine (1×10^{-6} M) to the superfusing fluid. The line on each trace represents the maximum diastolic potential before vagal stimulation.

progressively fell. Slowing was associated with a small increase in peak diastolic potential but the shape of the action potential was unchanged. The most marked change was a fall in the rate of diastolic depolarization. In some cells the threshold potential for the initiation of action potentials increased slightly (see as example Fig. 6*A*). Qualitatively similar records have been obtained from pacemaker cells of the toad sinus venosus (Bywater *et al.* 1989). In that tissue it was suggested that vagally released acetylcholine caused bradycardia by suppressing inward current flow during diastole.

Effects of vagal stimulation on action potentials recorded from driven cells

As with the recordings from pacemaker cells, the rate of generation of action potentials in driven cells fell during low-frequency vagal stimulation. With higher

frequency stimulation action potential generation ceased (Fig. 4). Again, when the heart was stopped, the membrane potential settled to a value positive to both the maximum diastolic potential and the potential at which 'driven' action potentials were normally initiated. Unlike the recordings from pacemaker cells after the end of

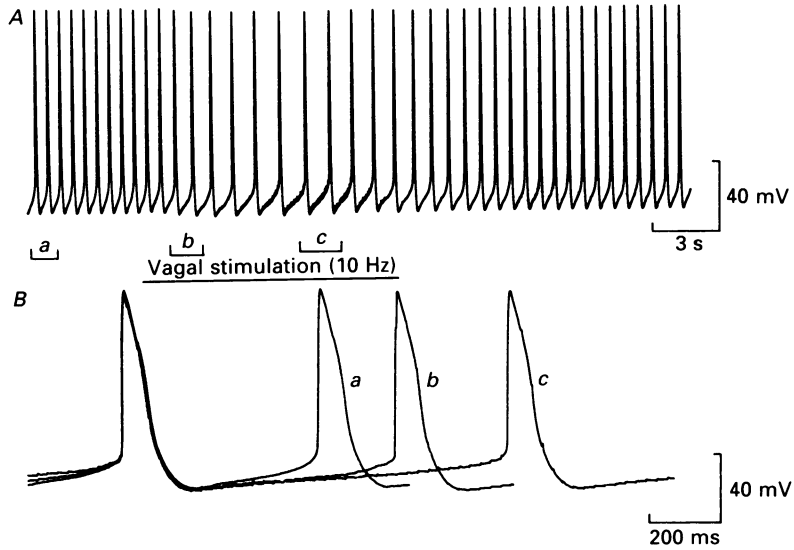

Fig. 3. Detailed changes in membrane potential observed during vagal slowing, recorded from a pacemaker cell. In *A*, displayed on a slow time base, the membrane potential of a pacemaker cell is shown before, during and after a 10 s period of vagal stimulation (stimulation frequency 10 Hz). *B* shows three expansions of the continuous trace taken from the parts of *A* underlined with continuous bars. The record *a* is from the control and the records *b* and *c* are during the stimulation period. It can be seen that the rate of diastolic depolarization had been slowed but the time courses and amplitudes of the action potentials were barely changed. The upper calibration bars refer to the upper record; the lower bars refer to each of the three expansions.

the stimulation period, when action potentials were again initiated there was no increase in the maximum diastolic potential nor was the rate of diastolic depolarization changed (see Fig. 4). As with pacemaker cells, the effects of vagal stimulation on driven cells were prevented by hyoscine.

Comparison of the effects of vagal stimulation and applied acetylcholine on pacemaker cells

Acetylcholine, added to the superfusion fluid in the concentration range 10^{-7}–10^{-4} M, caused changes in both the shapes of the pacemaker action potentials and their rates of occurrence. The lower concentrations had little effect on the frequency but both reduced the amplitude and shortened the time course of individual action potentials; their durations at half-maximum amplitude fell from about 100 ms to less than 15 ms. Higher concentrations ($> 1 \times 10^{-6}$ M), as well as shortening the duration of the action potentials, decreased their frequency and ultimately prevented the

generation of action potentials. An experiment is illustrated in Fig. 5. In the first part of this experiment the vagus was stimulated at 30 Hz for 20 s. The generation of action potentials stopped and the membrane potential settled to a potential positive of the maximum diastolic potential (Fig. 5*A*). In the second part of the experiment, with the electrode still in the same cell, a very high concentration of acetylcholine

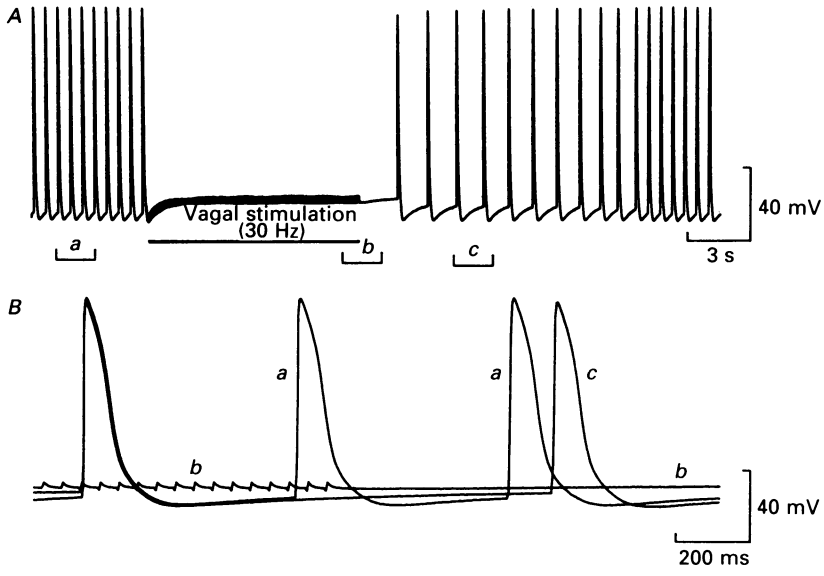

Fig. 4. Effect of vagal stimulation on the membrane potential of a driven cell. In *A*, displayed on a slow time base, the membrane potential of a 'driven' cell is shown before, during and after a 10 s period of vagal stimulation (stimulation frequency 30 Hz). Even during recovery from cardiac arrest, no increase in diastolic potential was detected in these cells. The expansions shown in *B* are taken from the control period marked *a*, and during (*b*) and after (*c*) vagal stimulation. It can be seen that there is little change in either the configuration of the action potential or the peak diastolic potential. After the period of vagal stimulation the rate of diastolic depolarization was not changed. The upper calibration bars refer to the upper record; the lower bars refer to each of the three expansions.

(1×10^{-4} M) was added to the perfusion fluid (Fig. 5*B*). It can be seen that again the generation of action potentials ceased but now the membrane potential settled to a more negative membrane potential than the control maximum diastolic potential. A high concentration of acetylcholine was used in this experiment so that an appreciable concentration of acetylcholine would rapidly reach the pacemaker cells of the sino-atrial node. In other experiments, lower concentrations (1×10^{-5} M) of acetylcholine were used and although they also stopped the pacemaker, the onset of action was much slower. We assume that the time delay reflects the mixing time within the recording chamber and the time taken for acetylcholine to diffuse to the pacemaker region. In this group of experiments the mean increase in membrane potential, compared to the maximum diastolic potential measured in control solution, during complete inhibition by superfused acetylcholine was $9{\cdot}3 \pm 1{\cdot}1$ mV ($n = 8$). These values correspond to absolute membrane potentials of about -75 mV.

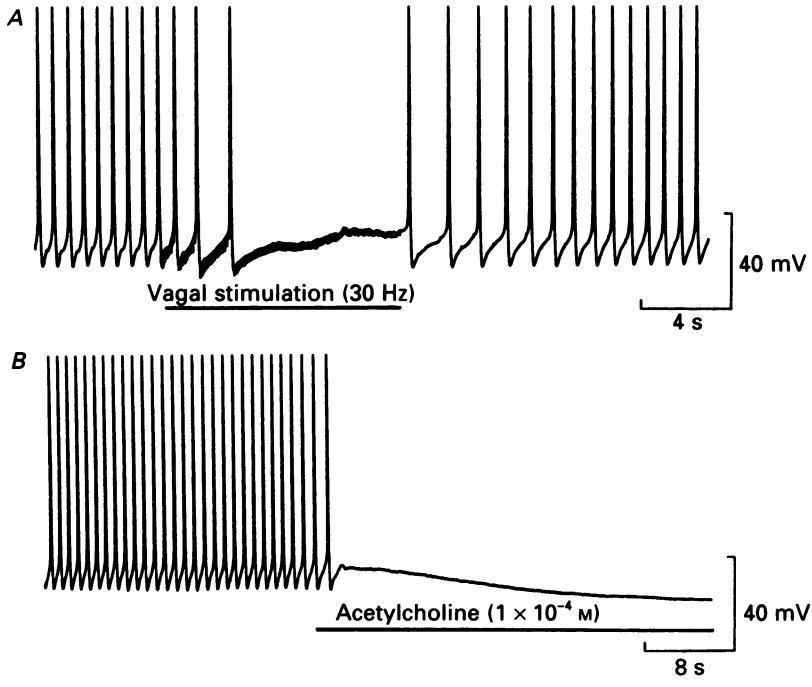

Fig. 5. Comparison of the effects of vagal stimulation and the effects of added acetylcholine. In the record shown in *A*, the vagus was stimulated at 30 Hz for 10 s and the spontaneous generation of action potentials stopped. Note that during the cessation of the beat the membrane potential settled to a value positive of the peak diastolic potential. The trace shown in *B*, recorded from the same cell as shown in *A*, is before and during superfusion with physiological saline containing acetylcholine (1×10^{-4} M). The membrane hyperpolarized and the generation of action potentials stopped.

The differences between the mechanisms of action of vagal stimulation and added acetylcholine were further illustrated by comparing the effects of low-frequency vagal stimulation with the onset of action of added acetylcholine. A comparison is shown in Fig. 6. In the left-hand part of the figure the effect of vagal stimulation (5 Hz for 10 s) is shown. As pointed out previously, vagal inhibition was associated with a decreased rate of diastolic depolarization (Figs 3 and 6*A* and *B*). In contrast, when added acetylcholine produced a similar degree of slowing, the peak diastolic potential was more negative and the action potential duration and amplitude were reduced (Fig. 6*C* and *D*). As in the toad, action potentials recorded in the presence of added acetylcholine were seen to be initiated at quite negative membrane potentials (for further discussion see Bywater *et al.* 1989). Each of the effects of added acetylcholine was prevented by the prior addition of hyoscine (1×10^{-6} M).

DISCUSSION

Low-frequency vagal stimulation slowed the heart by causing a small increase in peak diastolic potential and by reducing the rate of diastolic depolarization. During vagal slowing, the shapes of action potentials were barely affected. Higher-frequency

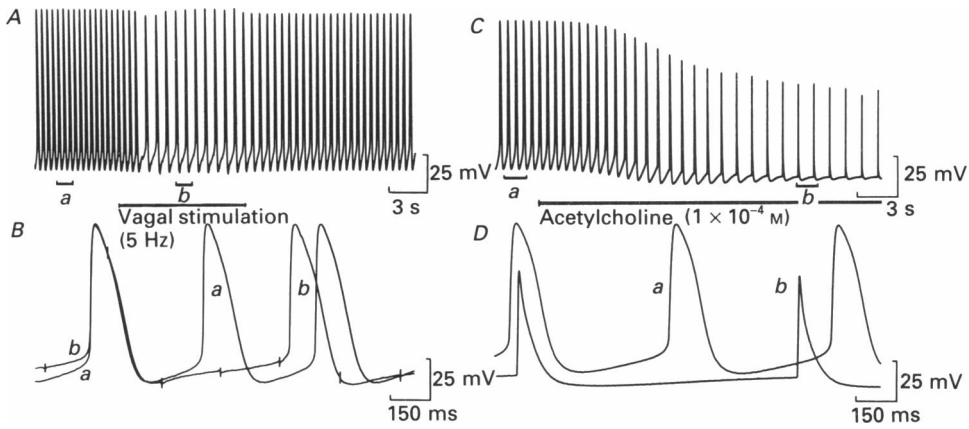

Fig. 6. Comparison of effects of low-frequency vagal stimulation and added acetylcholine on cardiac action potentials recorded from a pacemaker cell. The upper trace (*A*) shows a continuous membrane potential record before, during and after a 10 s train of vagal stimuli delivered at 5 Hz. Again it can be seen that during the period of vagal stimulation (underlined) the peak diastolic potential was little changed and only a slight transient decrease in the peak amplitude of the action potentials occurred. After the end of the train of stimuli the rate of generation of action potentials returned towards its control value. Part *B* of the figure shows two expansions of the continuous trace taken from record *A*. Record *a* is from the control and *b* is during the stimulation period. The rate of diastolic depolarization had slowed and the threshold depolarization for the initiation of the rapid upstroke moved to a more positive potential. The time course and amplitude of the action potentials was not changed. Part *C* shows a continuous membrane potential record obained before and during the addition of acetylcholine (1×10^{-4} M). It can be seen that during the application of acetylcholine (underlined) the peak diastolic potential was more negative and the peak amplitude of the action potentials was depressed. Part *D* shows two expansions taken from record *C* from the points underlined and labelled as *a* (control) and *b* (in the presence of acetylcholine). In the presence of acetylcholine the action potentials had brief time courses and reduced amplitudes.

vagal stimulation prevented pacemaker action potentials and the membrane potential settled to a potential positive of the maximum diastolic potential. Each of these effects was prevented by hyoscine: presumably neuronally released acetylcholine was activating muscarinic receptors to cause bradycardia. Added acetylcholine also produced slowing and cardiac arrest by activating muscarinic receptors. However, the membrane potential changes recorded during the addition of acetylcholine were different to those recorded during vagal stimulation. Added acetylcholine both shortened the duration of cardiac action potentials and caused a large increase in their peak diastolic potential. Again the effects of added acetylcholine were prevented by hyoscine.

Our observations on the effects of added acetylcholine are consistent with the view that it increases g_K in cardiac muscle cells. Many reports show that such an increase in g_K occurs (Hutter, 1957; Trautwein, 1963; Noble, 1975; Löffelholz & Pappano, 1985). Added acetylcholine activates muscarinic receptors, which in turn leads to the opening of a distinct set of K^+-selective channels (Sakmann, Noma & Trautwein, 1983). The increase in g_K caused by the activation of K^+-selective channels by added acetylcholine is voltage dependent: at hyperpolarized potentials the increase in g_K is

more marked than at depolarized potentials (Noma & Trautwein, 1978; Sakmann *et al.* 1983; Simmons & Hartzell, 1987). Nevertheless the increase in g_K continues to shorten the duration of action potentials even at membrane potentials positive of 0 mV (Iijima, Irisawa & Kameyama, 1985; Opthof, VanGinneken, Bouman & Jongsma, 1987).

The effects of vagally released acetylcholine are apparently quite different from those of applied acetylcholine. To explain the effects of the vagally released acetylcholine in terms of the opening of a set of K^+-selective ion channels, one would have to suggest that they have very unusual properties. The rectifying properties of the channels activated by neurally released acetylcholine would have to be different to those of the channels readily activated by added acetylcholine. They would have to produce an increase in conductance over a very restricted membrane potential range. Little current must flow at potentials negative of the maximum diastolic potential or positive of the threshold for the initiation of an action potential. To date chemically activated channels which allow current flow over such a limited membrane potential range have not been described.

The major effect of vagal stimulation on the pacemaker action potentials was to slow the rate of diastolic depolarization. Neuronally released acetylcholine would cause slowing if it changed the balance between inward and outward current flow during the diastolic period. That is, neuronally released acetylcholine might change the properties of channels which are activated normally in this limited membrane potential range. A number of distinct currents are thought to flow during diastole. There is a time-dependent decline of an outward potassium current (i_K, Brown, 1982); the channels for this current are activated during the action potential. During the repolarization phase of the action potential a set of channels are activated by hyperpolarization and these may provide an inward current, i_f, during diastole (Brown, 1982; DiFrancesco, 1985). During the diastolic depolarization a set of calcium channels ($I_{Ca,T}$ channels) are activated and these transiently allow the influx of calcium ions (Hagiwara, Hiroshi & Kameyama, 1988).

It seems unlikely that neurally released acetylcholine acts to simply increase i_K during diastole. Little change in the peak diastolic hyperpolarization was detected. However, it could be that the time-dependent closure of the channels responsible for i_K was slowed. The peak diastolic potential would be little affected but the outward current during diastole would be prolonged and the rate of diastolic depolarization slowed. An action potential would be initiated when a number of channels, similar to that occurring in the absence of vagal stimulation, had closed. The shape of a 'slowed' action potential would be similar to that of a 'control' action potential. If the rate of closure of the channels became very slow the membrane potential eventually would fail to reach threshold for the initiation of an action potential. During diastole the efflux of potassium would be more prolonged and the extracellular concentration of potassium would increase (see Spear *et al.* 1979). However, we are not aware of any indications that the channels responsible for the time-dependent potassium current, i_K, are affected by acetylcholine in this way.

Similarly our observations on the effects of vagal stimulation could be explained if neuronally released acetylcholine reduced inward current flow during the diastolic depolarization. For example, neuronally released acetylcholine might reset the

activation potential for the channels activated by hyperpolarization to a more negative potential. Normally these channels are activated at membrane potentials negative of −40 mV. As a result, the current, i_f, that they supply tends to move the membrane potential back to a depolarized level and so another action potential is generated (Brown, 1982; DiFrancesco, 1985). If during vagal stimulation the channels were only activated at a more hyperpolarized potential they would supply less depolarizing current. A slight increase in the maximum diastolic potential would occur since inward current flow during diastole would be reduced. The slope of the diastolic depolarization would decrease and the rate of generation of action potentials would be slowed. The threshold of the action potentials may increase because the net inward current at a given potential would be reduced. In the absence of action potentials the membrane potential would settle to the changed activation potential of i_f. However, since the membrane properties at potentials positive of the activation potential of i_f would not have been changed, there would be no change in the shape of action potentials (for further discussion see Bywater *et al.* 1989). An effect of acetylcholine, identical to this, has recently been demonstrated by DiFrancesco & Tromba (1987) using single isolated pacemaker cells from the rabbit in which both calcium and potassium channels had been blocked.

Whichever way vagally released acetylcholine causes bradycardia, the mechanism of vagal inhibition in the guinea-pig appears to be the same as that found in the toad. In the toad vagal stimulation continues to slow the heart after the effects of a muscarinic receptor-mediated increase in g_K have been blocked by barium ions (Momose, Giles & Szabo, 1984; Bywater *et al.* 1989). Furthermore in the toad vagal stimulation is in part mimicked by the addition of caesium ions which prevent the activation of i_f (DiFrancesco, Ferroni, Mazzanti & Tromba, 1986). These observations led to the suggestion that vagally released acetylcholine activates junctional muscarinic acetylcholine receptors (Bywater *et al.* 1989) that alter the activation voltage for i_f (DiFrancesco & Tromba, 1987).

In the guinea-pig, as with the toad, the effects of vagal stimulation were not closely mimicked by adding acetylcholine to the superfusion fluid. Yet both effects were prevented by a muscarinic antagonist. This suggests that there is some synaptic specialization between vagal postganglionic fibres and sino-atrial node cells of the guinea-pig. The muscarinic receptors by which applied acetylcholine increases g_K have been shown to be distributed broadly over the surfaces of cardiac muscle cells (Hartzell, 1980). Clearly the acetylcholine released from neurones is activating another set of muscarinic receptors, linked to different membrane mechanisms. Thus it appears that neurally released acetylcholine acts on specialized junctional receptors while applied acetylcholine acts on more widely distributed extrajunctional receptors.

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