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SYNAPTIC EXCITATION AND INHIBITION RESULTING FROM DIRECT ACTION OF ACETYLCHOLINE ON TWO TYPES OF CHEMORECEPTORS ON INDIVIDUAL AMPHIBIAN PARASYMPATHETIC NEURONES

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SUMMARY

1. Synaptic transmission was studied in visually identified parasympathetic ganglion cells that modulate the heart beat of the mudpuppy *Necturus maculosus*).

2. The brief pulse of acetylcholine (ACh) released from terminals of the vagus nerve after each impulse can produce two distinct post-synaptic responses in individual principal cells of the ganglion: (i) within a millisecond of release, ACh generates a rapid and strong excitatory post-synaptic potential (e.p.s.p.) that normally initiates a post-synaptic impulse; (ii) this excitation is usually followed by a slow hyperpolarizing inhibitory post-synaptic potential (i.p.s.p.) that lasts for several seconds. The magnitude and time course of the i.p.s.p. depends on the frequency and number of vagal stimuli. When the hydrolysis of ACh is inhibited by prostigmine, a train of nerve stimuli may be followed by an i.p.s.p. lasting half a minute or longer.

3. The rapid e.p.s.p. and slow i.p.s.p. result from the direct action of ACh on two different types of chemoreceptors in the post-synaptic membrane of the principal cell. The e.p.s.p. can be preferentially blocked by the nicotinic antagonist dihydro-β-erythroidine (5×10^{-7} M), while the i.p.s.p. is selectively blocked by the muscarinic antagonist atropine (5×10^{-9} M).

4. Potentials resembling nerve-evoked e.p.s.p.s and i.p.s.p.s can be produced by iontophoretic release of ACh from micropipettes onto the post-synaptic membrane. Application of the muscarinic agonist bethanechol generates exclusively inhibitory responses.

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5. The reversal potential for the i.p.s.p. is about -105 mV, which is approximately the equilibrium potential for potassium (E_K). When the external K^+ concentration is altered, the reversal potential for inhibition is shifted to the new value of E_K as expected from the Nernst equation. Changes in the external Na^+ and Cl^- concentrations have no appreciable effect on the reversal potential. Thus, the i.p.s.p. is the result of a conductance increase for K^+.

6. The conductance change producing the i.p.s.p. is voltage sensitive. When the membrane potential is shifted from -40 to -60 mV, the i.p.s.p becomes larger and longer. Beyond -60 mV the inhibitory response decreases in proportion to the driving force on K^+ without any further change in time course.

7. The inhibitory response produced by an iontophoretically applied pulse of bethanechol has a delayed onset of about 150 msec at 24 °C. The early portion of this response, including the delay, is proportional to t^3, where t is time. The proportionality factor (the apparent rate constant) decreases elevenfold when the temperature is lowered by 10 °C. This suggests that a multi-step process is involved in the activation of the conductance increase that leads to the inhibitory response. Inhibitory responses with similar kinetics were produced in heart muscles of the mudpuppy upon application of ACh.

INTRODUCTION

The details of chemical synaptic transmission are best understood at vertebrate skeletal neuromuscular synapses (for reviews see Katz, 1966; Hubbard, 1973; Krnjević, 1974). In these, the release of acetylcholine (ACh) from a motor nerve terminal gives rise to a rapid excitatory post-synaptic potential (e.p.s.p.) that initiates a conducted muscle impulse. The total duration of the e.p.s.p. is about 30 msec. The processes of chemical transmission are not as well understood in autonomic ganglia, where synaptic responses may last seconds or even minutes. For example, in neurones of sympathetic ganglia, stimulation of a presynaptic nerve causes an initial fast e.p.s.p., which is mediated by ACh and is analogous to that at the skeletal neuromuscular synapse. Following this, however, there are a variety of slower potentials, some excitatory, others inhibitory (for reviews see Libet, 1970; Nishi, 1974; Volle, 1975). Sympathetic ganglia also possess catecholamine-containing interneurones which are thought to produce the slow inhibitory post-synaptic potentials (i.p.s.p.s) (Eccles & Libet, 1961; Libet, 1970).

We have chosen to analyse synaptic transmission in an amphibian parasympathetic ganglion which innervates the heart of the mudpuppy

(*Necturus maculosus*). This ganglion is embedded in a thin transparent sheet of tissue in which the cellular components can be viewed in great detail, as originally described by McMahan & Purves (1976). Excitatory synaptic transmission in the principal cells of this preparation is mediated by ACh (Roper, 1976*a*). These ganglia also have catecholamine-containing interneurones analogous to those of mammalian sympathetic ganglia (McMahan & Purves, 1976). We therefore thought that these interneurones might contribute slow inhibitory potentials. It turned out that we did observe slow i.p.s.p.s in the principal cells. They are not, however, caused by interneurones, whose function remains unknown; rather, the i.p.s.p.s result from a direct effect of ACh released by the vagus nerve on to principal cells.

Thus, nerve-released ACh causes first a rapid and powerful excitatory synaptic potential with a total duration of about 50 msec and subsequently a much slower i.p.s.p. lasting seconds. The same transmitter, ACh, combines with two distinct chemoreceptors on the same cell. Activation of 'nicotinic' receptors gives rise to the e.p.s.p., whereas 'muscarinic' receptors are responsible for the i.p.s.p.

METHODS

Preparation. Anatomical and physiological properties of the parasympathetic cardiac ganglion in the mudpuppy have been described previously (McMahan & Purves, 1976; Roper, 1976*a*, *b*). The neurones in this preparation lie in a thin sheet of connective tissue so that considerable subcellular detail, including the sites of synaptic boutons, can be resolved in the isolated living preparation with differential interference (Nomarski) optics. There are two classes of neurones in this preparation: large principal cells, 30–50 μm in diameter, and smaller, catecholamine-containing cells, about 15–30 μm in diameter (Fig. 1). The principal cells are analogous to their counterparts in the parasympathetic cardiac ganglion of the frog (Dennis, Harris & Kuffler, 1971) and receive a cholinergic excitatory synaptic input from the vagus nerve (Roper, 1976*a*). Their membrane potentials were usually between -40 and -60 mV. The physiological properties of the smaller interneurones are largely unknown except that they produce action potentials upon direct depolarization (Roper, 1976*a*). Further, they are depolarized by iontophoretic application of ACh and excited by vagal stimulation (H. C. Hartzell, S. W. Kuffler & D. Yoshikami, unpublished). It is not known whether this vagal stimulation acts monosynaptically. The interneurones have the characteristic catecholamine fluorescence when fixed by the Falck–Hillarp technique (McMahan & Purves, 1976). A very convenient procedure for producing the catecholamine fluorescence was to fix the whole mount with 1% glyoxylic acid (dissolved in 75 mM-sodium phosphate; final pH, 7·0), as described by Lindvall & Björklund (1974). For our preparation this histochemical fluorescence technique provided results comparable to those produced by the method of Falck & Owman (1965). Biochemical assays indicate that most of the catecholamine in this preparation is dopamine (R. Stickgold, unpublished). For physiological examination, the tissue was prepared as previously described (Roper, 1976*a*; see also Dennis *et al.* 1971).

Solutions. The volume of the bathing solution was less than 0·3 ml. and was continuously exchanged at rates varying from 0·05 to 6 ml./min. Ringer solutions normally contained (mM): NaCl, 115; $CaCl_2$, 3·6; and KCl, 2·0; buffered at pH 7·0 with 1 mM-Na-HEPES (Na-*N*-2-hydroxyethylpiperazine-*N'*-ethane sulphonate). When catecholamines were perfused, the Ringer solutions were supplemented with

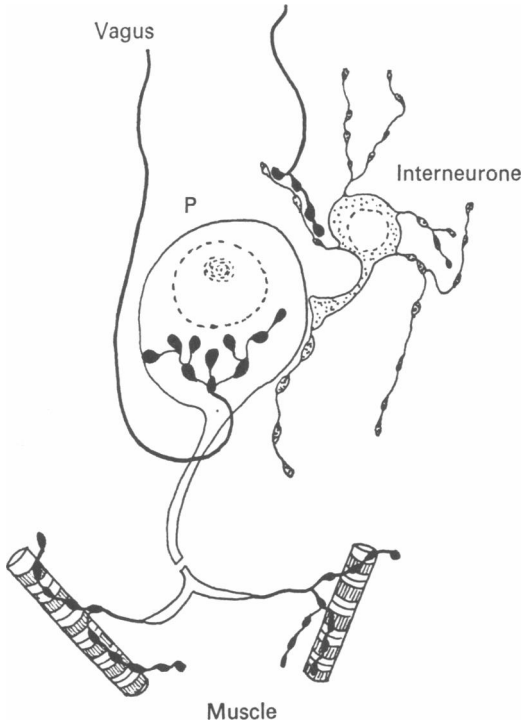

Fig. 1. Sketch of the parasympathetic cardiac neurones and their afferent and efferent connections. A principal cell (P) and a small, catecholamine-containing interneurone are shown. The principal cells (and probably the interneurones, see text) receive direct cholinergic input from the vagus nerve. The principal cells have processes that release ACh on to heart muscle, where it has an inhibitory action. Anatomical studies show an occasional synaptic contact of interneurones with principal cells. (Drawing kindly provided by U. J. McMahan; after McMahan & Purves, 1976; Roper, 1976*a*).

10% (v/v) Leibowitz medium (*cf.* Dennis *et al.* 1971; Roper, 1976*a*), which retards oxidation of the catecholamines (R. Stickgold, unpublished). The catecholamines were dissolved in this solution and used within 30 min. Control solutions also contained Leibowitz medium. The bath perfusion rate in these experiments was about 6 ml./min.

Experiments were normally done at room temperature (22–24 °C). In some tests the preparation was perfused with chilled Ringer solution, and temperature was

monitored with a 200 μm diameter thermocouple probe placed within 1 mm of the water-immersion objective.

Electrical recording. Glass micro-electrodes were pulled on a conventional horizontal puller from capillaries containing a glass fibre. They were filled by injecting 3 M-KCl into the shaft and had resistances of 100–200 MΩ. Iontophoretic pipettes contained 2 M-ACh chloride or 2 M-bethanechol (carbamyl-β-methylcholine chloride) and had resistances of about 100 MΩ. Conventional recording and iontophoretic techniques were used as previously described (Dennis *et al.* 1971; Kuffler & Yoshikami, 1975; Roper, 1976*a*). Braking currents of about 4 nA were applied to the iontophoretic pipettes and their tips were positioned so that under high magnification (400 ×) they were seen to touch the neuronal surface.

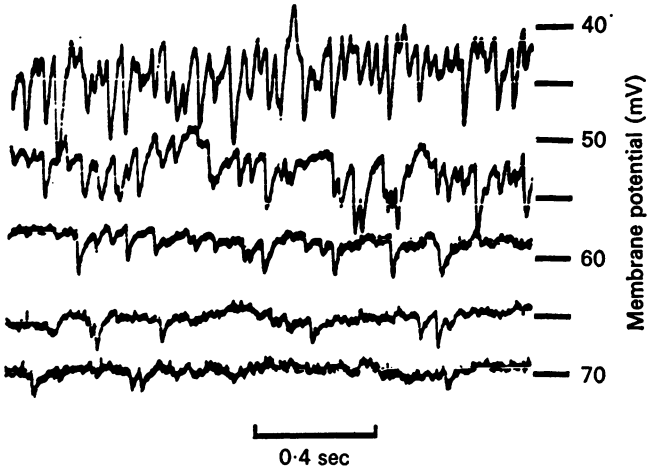

Fig. 2. Spontaneous hyperpolarizing potentials in principal cells of the parasympathetic cardiac ganglion. The membrane potential was monitored with one intracellular electrode while a second intracellular current-passing electrode adjusted the membrane potential to different levels. The spontaneous hyperpolarizations have amplitudes near 5 mV at the resting potential (−43 mV). They decrease to less than 1 mV when the cell is hyperpolarized to −70 mV. The spontaneous hyperpolarizations are thought to be due to fluctuations in K^+ conductance. They appear on many of the subsequent records.

In virtually every principal cell one recorded a conspicuous membrane 'noise' that appeared as brief, spontaneous hyperpolarizing potentials of a few millivolts. These hyperpolarizations are sensitive to the membrane potential, and when the cell is hyperpolarized, their amplitudes decrease (Fig. 2). Although these fluctuations have not been studied in detail, they are thought to arise from spontaneous changes in K^+ conductance (unpublished). They have previously been seen by S. Roper (private communication) and are mentioned here because they are evident in several of the records to be presented (e.g. Figs. 4, 5 and 11).

RESULTS

Nerve-evoked excitatory and inhibitory responses

When one records with an intracellular electrode from principal cells in the cardiac ganglion and stimulates the vagus nerve with a single stimulus, one typically observes a sequence of potentials, as illustrated in Fig. 3. The transmitter, released from the synaptic boutons of vagal nerve terminals, causes a rapidly rising e.p.s.p. that usually initiates a conducted

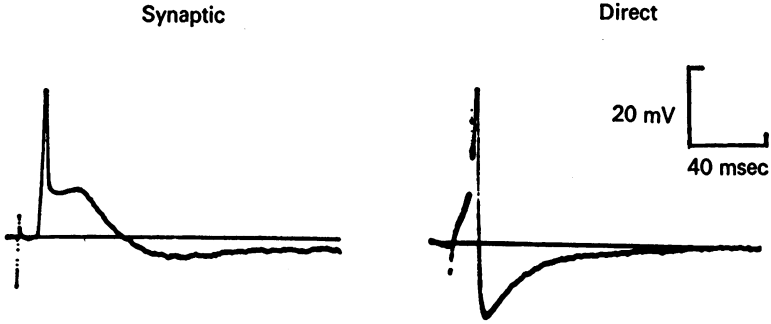

Fig. 3. Intracellular records from a principal cell in the parasympathetic cardiac ganglion. *Left*: a single stimulus to the vagus nerve typically causes a rapidly rising e.p.s.p. that initiates an impulse. This is followed by a depolarization that represents the continued action of the transmitter, ACh. *Right*: direct electrical stimulation by a current pulse through the recording electrode also initiates an impulse followed by an undershoot.

post-synaptic impulse in less than 1 msec. This is followed by a slower depolarization resulting from the continued action of the transmitter. The subsequent hyperpolarization is due almost entirely to the undershoot of the action potential, although a small inhibitory potential (see below) may also contribute to the response. As a rule the entire sequence does not last longer than 150 msec. In comparison, when the principal cell is stimulated directly, either by an electric current passed through the recording electrode or by an antidromic impulse, one sees a simpler signal that lacks the contribution of the transmitter (Fig. 3 and Dennis *et al.* 1971; Roper, 1976*a*).

When the vagus nerve is excited by a train of stimuli, as in Fig. 4, a corresponding train of post-synaptic impulses results, typically followed by a slow hyperpolarization that persists for several seconds. As the stimulation frequency is increased (e.g. from 10 to 30/sec), larger and longer responses are produced (Fig. 4). Such hyperpolarizations do not occur when neurones are excited by direct electrical stimulation, and are therefore the result of the action of a synaptic transmitter. This finding,

together with evidence to be presented below, indicates that the hyperpolarization which arises after nerve stimulation can be classified as an inhibitory post-synaptic potential (i.p.s.p.).

On a few occasions an i.p.s.p. could be detected when only a single stimulus was applied to the vagus (not shown). Normally, however, such responses were so small that they had to be signal-averaged to be seen clearly. This indicates that the i.p.s.p. produced in response to a train of

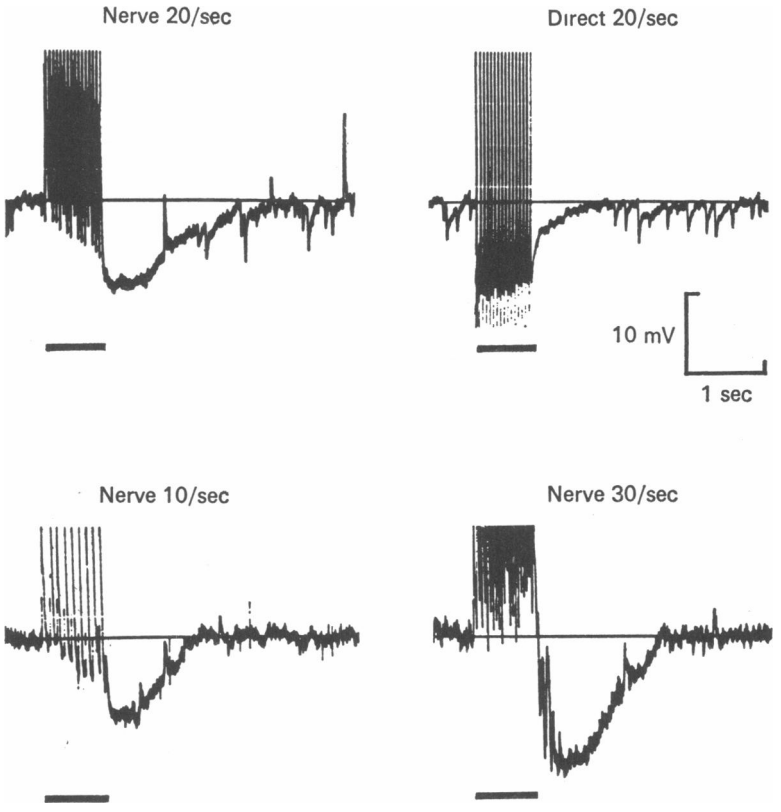

Fig. 4. Nerve-evoked excitatory and inhibitory responses. *Top traces*: 16 stimuli at 20/sec to the vagus nerve caused conducted impulses (peaks cut off), followed by an inhibitory hyperpolarization (i.p.s.p.) which lasted about 2 sec. The same number of impulses produced by direct electrical stimulation through the recording electrode in this neurone did not initiate a similar i.p.s.p. *Lower traces*: another cell when the vagus was stimulated for 0·8 sec at 10 and 30/sec. The amplitude and duration of the i.p.s.p.s varied with the frequency and number of nerve stimuli. Note the rapid depolarizations, representing delayed release of quanta, following the cessation of the trains of nerve stimuli. The rapid downward deflections are spontaneous hyperpolarizations (see Fig. 2).

vagal stimuli is a summation of responses with a contribution from every stimulus. In other tests it was shown that e.p.s.p.s and i.p.s.p.s were produced at the same sharp threshold of stimulation, suggesting that excitation of a single vagal axon can produce both responses. In some cells we could not observe i.p.s.p.s, although the neurones appeared in good condition, as judged by vagally evoked e.p.s.p.s that initiated overshooting action potentials. We have not specifically studied this variability in responses.

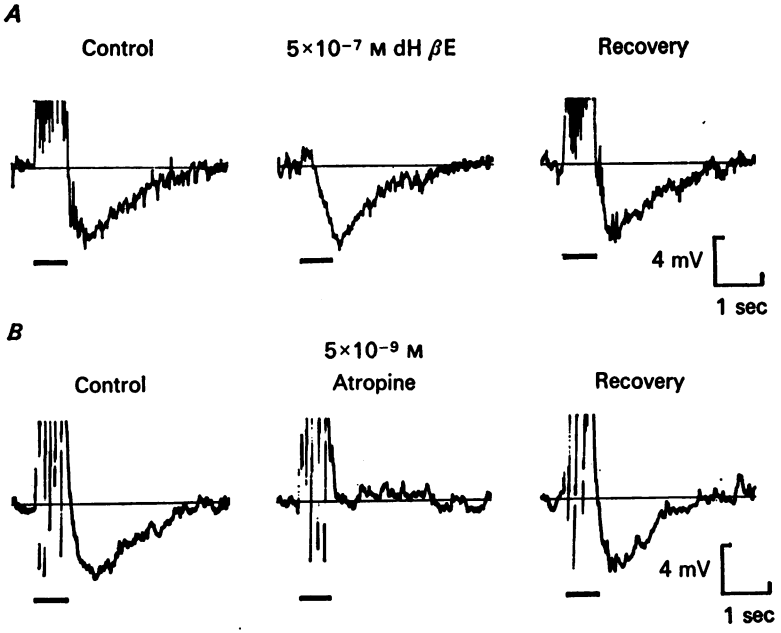

Fig. 5. Separation of excitatory and inhibitory responses by cholinergic antagonists. *A*, records from a principal cell stimulated by fifteen vagal stimuli at 30/sec. Bath application of the nicotinic antagonist dihydro-β-erythroidine (dHβE) selectively abolishes the e.p.s.p.s and post-synaptic impulses. *B*, application of atropine, a muscarinic blocker, to the same neurone eliminates the i.p.s.p., leaving intact the e.p.s.p.s and concomitant action potentials (peaks cut off). The effects of both inhibitors were reversible.

Separation of excitatory and inhibitory responses

Since different ionic channels are activated in the production of the e.p.s.p. and i.p.s.p., it was expected that different receptors would be involved. The receptors responsible for the fast e.p.s.p. were known to have 'nicotinic' properties in view of their sensitivity to dihydro-β-erythroidine, a nicotinic antagonist (Roper, 1976*a*). On the other hand, the time course of the slow i.p.s.p. in the principal cells resembles that

produced by ACh application on to the heart muscle, which has 'muscarinic' receptors (see Fig. 14). Therefore, we investigated the effects of nicotinic and muscarinic antagonists on the synaptic responses of the principal cells.

The block of the e.p.s.p. by dihydro-β-erythroidine (5×10^{-7} M) was readily observed, while the i.p.s.p. was not appreciably affected (Fig. 5*A*). Conversely, when atropine (5×10^{-9} M), a muscarinic antagonist, was applied, only the i.p.s.p. was blocked, leaving the e.p.s.p. intact (Fig. 5*B*). With higher concentrations of dihydro-β-erythroidine (about 10^{-6} M) the i.p.s.p. was also reduced. Other nicotinic blockers, such as hexamethonium and curare, required higher concentrations (about 0·1 mM) and were not as specific in inhibiting only the excitatory responses.

These results lead to the conclusion that two types of ACh receptors are involved: a nicotinic receptor which mediates the e.p.s.p. and a muscarinic receptor which mediates the i.p.s.p. Additional experiments (below) show that both types of receptors are situated on principal cells and are directly activated by ACh released upon vagal stimulation.

Effect of anticholinesterase

The enzyme acetylcholinesterase normally hydrolyses ACh and thereby shortens the duration of its post-synaptic action. In the presence of the anticholinesterase prostigmine, the e.p.s.p. and i.p.s.p. are prolonged. In the example of Fig. 6 (control) a train of ten stimuli at 20/sec produced the usual burst of impulses in a principal cell, followed by an i.p.s.p. lasting about 2 sec (cf. Figs. 4, 5). In contrast, after treatment with prostigmine the same cell produced an i.p.s.p. whose duration was about 15 sec. A second cell in the same preparation showed an i.p.s.p. lasting over 35 sec (Fig. 6, bottom trace). In some other prostigmine-treated preparations, i.p.s.p.s had amplitudes of 30 mV and durations of 1 min or longer. In several of the tests an increased 'delayed' release of ACh was seen after trains of nerve stimuli (see Fig. 4), and this must have contributed to the prolonged potentials. The time course of the e.p.s.p.s also became longer after treatment with prostigmine, but to a lesser extent. This prolongation was best seen after the i.p.s.p.s had been blocked by atropine.

Excitatory and inhibitory potentials produced by iontophoretic application of cholinergic agonists

Responses to applied ACh. Excitatory post-synaptic potentials can be mimicked in principal cells by iontophoretically applying ACh (Roper, 1976*a*, *b*). If nerve-released ACh were directly responsible for the i.p.s.p. as well, one should be able to evoke the inhibitory response by applying ACh directly to the post-synaptic membrane. In agreement with this

hypothesis, iontophoretic application of ACh produced excitatory responses followed by inhibitory potentials. They could be evoked by either single or trains of ACh pulses. An example is shown in Fig. 7*A* and *C*. These records are analogous to the nerve-evoked synaptic potentials of Figs. 4 and 5. In addition, as expected, there was a selective block of the early rapid depolarization by dihydro-β-erythroidine (5×10^{-7} M, Fig. 7*B*) and of the hyperpolarization by atropine (5×10^{-9} M, Fig. 7*D*).

Response to bethanechol. Bethanechol (carbamyl-β-methylcholine), an analogue of ACh, is a well-established muscarinic agonist (Koelle, 1975*a*). In the example of Fig. 8 it was applied iontophoretically onto the surface

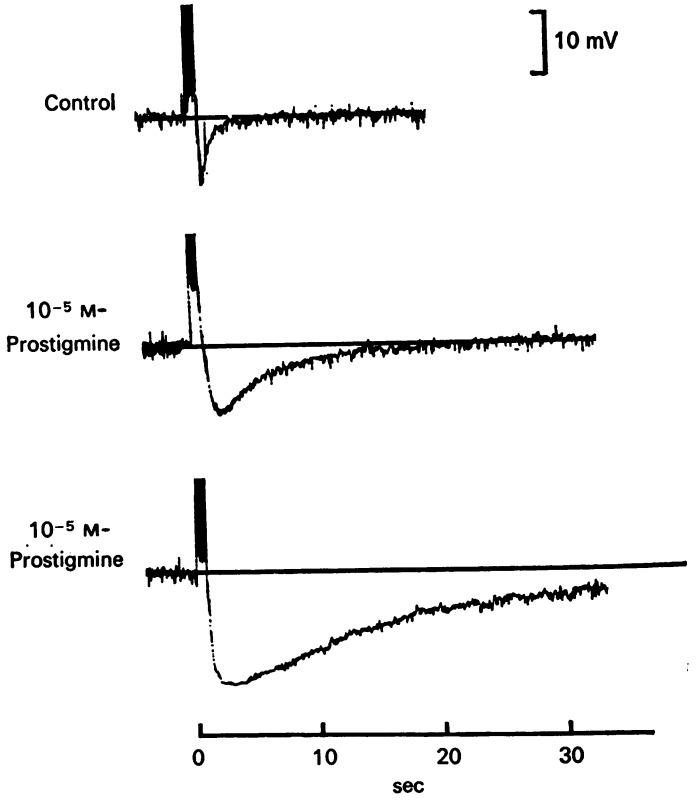

Fig. 6. Effect of the anticholinesterase, prostigmine, on the i.p.s.p. The response of a principal cell was recorded while the vagus nerve was stimulated for 0·5 sec at 20/sec. In the control, the usual e.p.s.p.s initiated action potentials (peaks cut off) followed by an i.p.s.p. lasting about 2 sec. When prostigmine was added to the bath (middle trace), e.p.s.p.s from the same cell were potentiated and the duration of the i.p.s.p. was prolonged to about 15 sec. The bottom trace shows a response from a second cell in the same preparation, whose i.p.s.p. lasted more than 35 sec.

of a principal cell. A 50 msec pulse produced a pure hyperpolarization lasting for about 4 sec and no trace of a depolarization could be detected. As expected, atropine (5×10^{-9} M) reversibly blocked the inhibitory potential. Conversely, the nicotinic antagonist dihydro-β-erythroidine (5×10^{-7} M) had no effect (see Fig. 7 *B*).

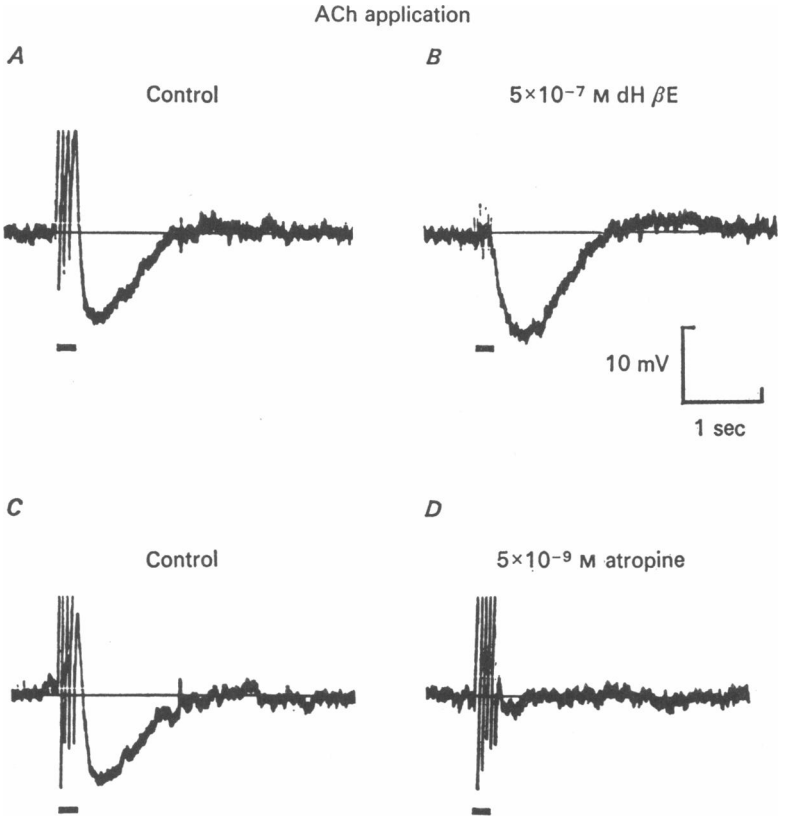

Fig. 7. Response to iontophoretically applied ACh. A 0·2 sec train of 1 msec current pulses at 30/sec released ACh on to the cell surface. *A* and *C*, ACh-evoked impulses (peaks cut off) are followed by a slow hyperpolarization which resembles the i.p.s.p. produced upon nerve stimulation, as in Figs. 4, 5, 6. *B*, bath application of dihydro-β-erythroidine (dHβE) specifically blocks the depolarizing response (see Fig. 5*A*). *D*, atropine inhibits only the hyperpolarizing response (as in Fig. 5 *B*). Both blocking effects are reversible. All responses are from the same cell.

The main differences between the inhibitory responses produced by bethanechol and ACh are: (i) much larger iontophoretic current pulses (tenfold or more) were required with bethanechol than with ACh to cause a similar inhibitory response, suggesting that bethanechol is a weaker

agonist (e.g. see Fig. 9); (ii) while the response produced by ACh was potentiated by the anticholinesterase Tensilon (edrophonium chloride), the bethanechol response was not affected. This is consistent with the fact that bethanechol is not hydrolysed by acetylcholinesterase (Koelle, 1975*a*).

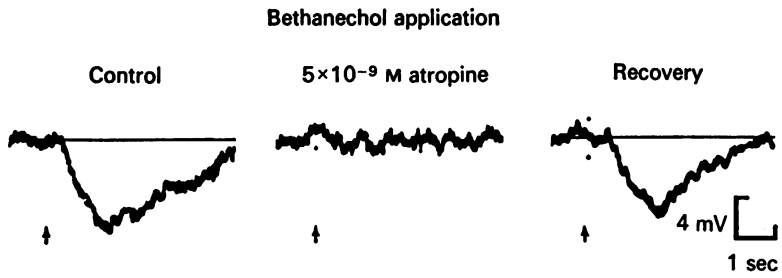

Fig. 8. Response to iontophoretically applied bethanechol. A 50 msec pulse of bethanechol caused, after a slight delay, a hyperpolarizing response lasting about 4 sec. The response was reversibly blocked by atropine, but not affected by 5×10^{-7} M-dihydro-β-erythroidine (not shown). Thus, bethanechol specifically activates the muscarinic receptors, in contrast to ACh which activates the nicotonic receptors as well (cf. Fig. 7).

Site of action of muscarinic agonists. It may still be argued that ACh and bethanechol do not act directly on the post-synaptic membrane of principal cells, but rather on nearby interneurones or their terminals (see Fig. 1). These, in turn, could release another transmitter, presumably a catecholamine, which would then cause the i.p.s.p. The involvement of such interneurones is excluded on several grounds. First, applied ACh still caused the inhibitory (as well as excitatory) responses even when release from nerve terminals was completely blocked by reducing the Ca^{2+} concentration in the bath to 0·1 mM and raising the Mg^{2+} concentration to 5 mM, or when transmission was prevented by 10 mM-Co^{2+}. Secondly, ACh and bethanechol were usually most effective in producing the inhibitory response when applied within a few micrometres of the principal cell, and therefore a primary effect on interneurones is unlikely. Thirdly, in several preparations there were areas in which no evidence of interneurones or their processes was observed by the histochemical fluorescence method of Lindvall & Björklund (1974; see Methods). Nevertheless, inhibitory responses were observed in principal cells within these areas.

An additional experiment tested the possible involvement of catecholamines in producing the i.p.s.p. The membrane potential of a principal cell was monitored while the bath was perfused with successive solutions of dopamine, norepinephrine, and epinephrine, each at a concentration of 0·1 mM (see Methods). None of these solutions had any measurable effect

on the membrane potential. To demonstrate that the bath-applied substance had ready access to cell surfaces, a fourth solution, containing bethanechol, was tested. In contrast to the catecholamines, a 0·1 mM solution of bethanechol immediately hyperpolarized the cell by 30 mV. Thus it is unlikely that the i.p.s.p. is mediated by a catecholamine.

We conclude that the i.p.s.p. is a result of the direct action of ACh on the post-synaptic membrane of principal cells.

The ionic basis of the inhibitory potential

In order to establish the ionic fluxes that account for the i.p.s.p., we determined the membrane potential at which the inhibitory response reversed its polarity, and studied the effect of different ion concentrations on the reversal potential.

Reversal potentials of the inhibitory response. As a first step, the reversal potential of the hyperpolarization resulting from nerve stimulation was compared with that produced by applied ACh and bethanechol. In this experiment dihydro-β-erythroidine (5×10^{-8} M) was added to the bathing solution in order to reduce the excitatory potentials evoked by nerve stimulation or applied ACh. All the tests were done on the same neurone. As illustrated in the sample records of Fig. 9*A*, when the membrane potential was displaced from -45 to -140 mV, the inhibitory response became progressively smaller and eventually reversed its polarity somewhere between -90 and -110 mV. A plot of the entire series of responses (Fig. 9*B*) shows that the reversal potential is near -105 mV. The nerve- and ACh-evoked excitatory responses progressively increased in amplitude as the neurone was hyperpolarized. The response to ACh application is shown by the dotted line. Extrapolation of this line (Fig. 9*B*) suggests that the reversal potential was near -25 mV. Nerve-evoked excitatory responses could not be measured because they were obscured by impulses. The membrane potential values in the experiment of Fig. 9 are only estimates, because the same electrode was used for recording and current passing, a procedure that may introduce errors. Further tests, using two intracellular electrodes (see below), confirmed the value of -105 mV for the reversal potential of the inhibitory response (see Table 1), while the excitatory response actually reversed at about -10 mV.

Regardless of the validity of the absolute values, since all measurements were done on the same neurone, the common reversal potential in Fig. 9 assures us that the same ionic mechanisms are involved in the inhibitory responses produced by nerve, ACh, and bethanechol.

Identification of the ions producing inhibition. Reliable determinations of the reversal potential for the i.p.s.p. were obtained when two intracellular electrodes were used, one to monitor the membrane potential

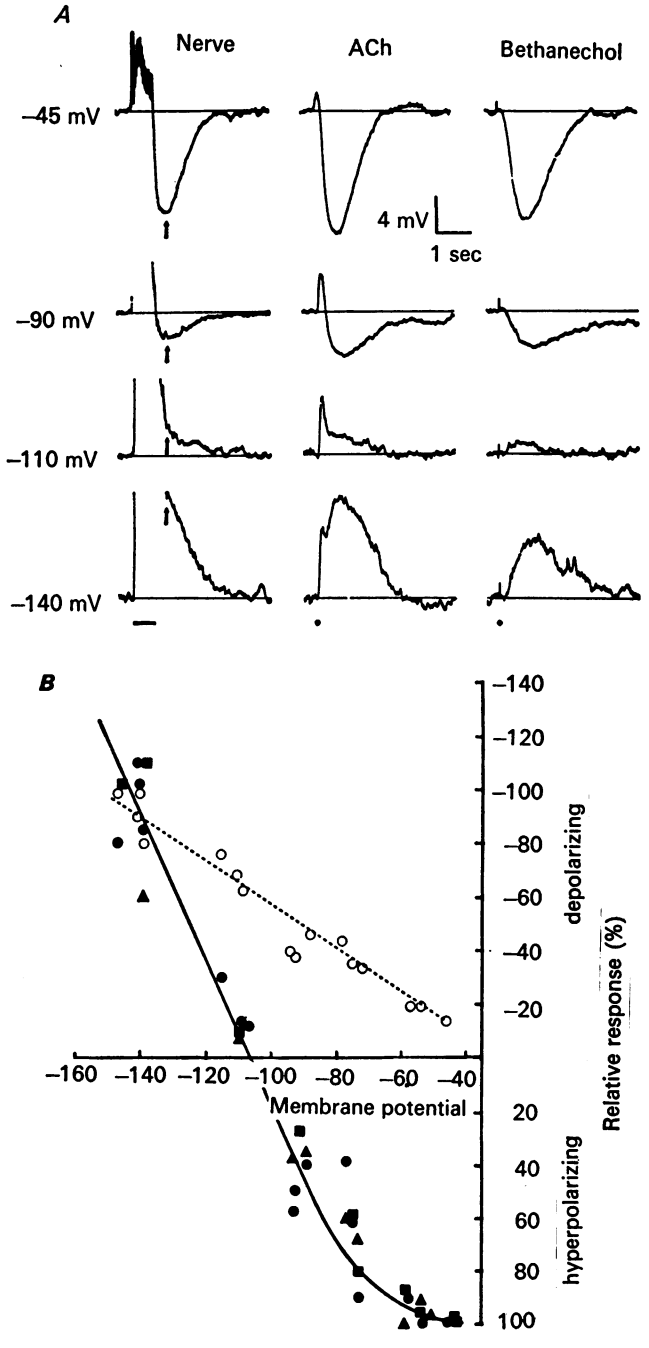

Fig. 9. For legend see opposite.

TABLE 1. The effects of external ion concentrations on the reversal potential of the inhibitory response. The membrane potential was shifted with a separate intracellular current electrode. Inhibitory responses were evoked by either ACh or bethanechol (BCh). When ACh was used, dihydro-β-erythroidine (5×10^{-7} M) and prostigmine (10^{-5} M) were present. [a]Sodium replaced with sucrose; [b]sodium replaced with Tris(hydroxymethyl)aminomethane; [c]chloride replaced by sulphate; averages given as mean values ± S.E. of mean; change in reversal potential per decade is obtained from

$$\frac{\text{change in reversal potential (mV)}}{\log_{10}(\text{fraction of normal concentration})}.$$

Ion Varied	Agonist	Reversal potential (mV)		Fraction of normal [ion]	Change in rev. pot. (mV)	Change in rev. pot. (mV) per decade
Potassium		Normal K^+	High K^+			
	ACh	−113	−73	5×	40	57
	ACh	−96	−54	5×	42	60
	BCh	−108	−63	5×	45	64
		−106 ± 6	−63 ± 8			61 ± 3
Sodium		Normal Na^+	Low Na^+			
	ACh	−102	−96[a]	1/5	6	−9
	ACh	−107	−107[a]	1/10	0	0
	BCh	−94	−86[a]	1/10	8	−8
	BCh	−114	−116[b]	1/100	−2	1
	BCh	−109	−111[b]	1/100	−2	1
		−105 ± 4	−103 ± 5			−3 ± 2
Chloride		Normal Cl^-	Low Cl^-			
	BCh	−105	−105[c]	1/14	0	0
All cells		−105 ± 2 (n = 9)				

Fig. 9. Reversal potentials of the inhibitory responses resulting from nerve stimulation (30/sec for 0·7 sec), iontophoretic application of ACh (20 nA for 10 msec) and bethanechol (170 nA for 10 msec). Membrane potential was monitored and varied by current passed through a single electrode. The bathing solution contained 5×10^{-8} M-dihydro-β-erythroidine to attenuate the excitatory responses. *A*, sample traces of the responses. As the cell was hyperpolarized, the fast excitatory responses became progressively larger, while the inhibitory responses became smaller and reversed their polarity between −90 and −110 mV for all modes of stimulation. The stimulus is indicated by a bar or dot. The excitatory responses to nerve stimulation partially obscured the inhibitory responses whose peaks are marked by arrows. *B*, plot of entire series. Inhibitory responses to nerve stimulation (■), ACh (●), and bethanechol (▲). Excitatory responses for ACh (○). Peak amplitudes were normalized to those at −45 mV and plotted as a function of membrane potential. All inhibitory reversal potentials are near −105 mV. Extrapolation of the depolarizing responses, plotted for ACh only, suggests a reversal potential near −25 mV.

and the other to pass current in order to depolarize or hyperpolarize the cell. With this method several experiments, using ACh as well as bethanechol as agonists, gave a mean value for the reversal potential of -105 mV (Table 1).

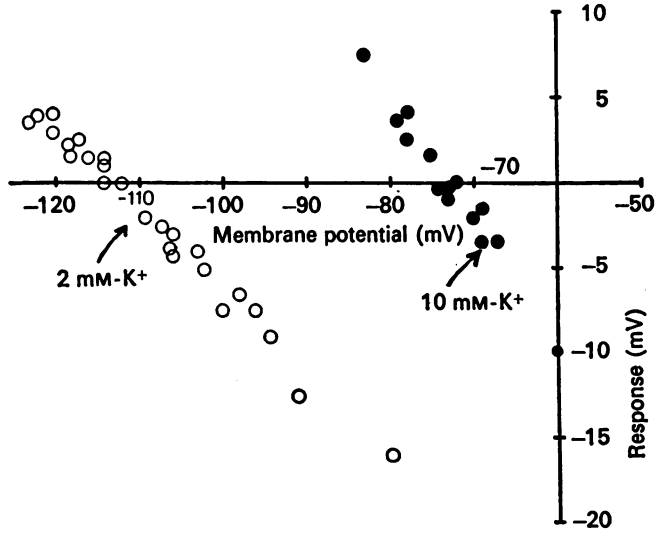

Fig. 10. Effect of changing $[K^+]_0$ on the reversal potential of the inhibitory response to ACh. The reversal potential was measured as in Fig. 9, except that two intracellular electrodes were used, one for recording voltage, and one for passing current to vary the membrane potential. The bath contained 10^{-5} M-prostigmine and 5×10^{-7} M-dihydro-β-erythroidine. In this example the reversal potential in normal Ringer solution (2 mM-K^+) was -113 mV, and it shifted to -73 mV when the bath contained 10 mM-K^+. Thus a change of 40 mV was produced by a fivefold change in $[K^+]_0$. See Table 1 for data from other experiments.

The ions that could have an equilibrium potential near -105 mV are K^+ and Cl^-. If K^+ were the sole responsible ion, the reversal potential of the i.p.s.p. would be identical with the equilibrium potential of K^+, E_K, and a change in the external K^+ concentration, $[K]_0$, should change the reversal potential, as predicted from the Nernst equation, which at 23 °C is

$$E_K = 59 \log \frac{[K]_0}{[K]_1}.$$

Thus, if $[K]_0$ were increased from 2 to 10 mM, one would expect the reversal potential to shift by 59 log 5, or 41 mV. In the example of Fig. 10 the shift was 40 mV, which is essentially the expected value. The results of several such experiments are summarized in Table 1.

In a complementary test, in which [Cl^-] in the bathing solution was reduced fourteenfold by substituting the impermeable sulphate ion, the reversal potential did not shift. Likewise, the reversal potential was not altered when sucrose or Tris was substituted for 80–99 % of the Na^+ in the Ringer solution. These results are included in Table 1 and indicate that changes in Cl^- and Na^+ conductances do not contribute significantly to the i.p.s.p.

E_K is frequently estimated from the reversal potential for the undershoot of the action potential. Since it was difficult to evoke impulses when the principal cells were hyperpolarized to -105 mV, the bathing K^+ was raised to 10 mM. Under these circumstances the reversal potential for the undershoot was readily measured, using two intracellular electrodes. The peak of the undershoot consistently reversed at a membrane potential about 3 mV less negative than that at which the inhibitory response reversed. We suspect that this slight difference is due to the presence of a residual Na^+ conductance during the peak of the undershoot.

We conclude that the inhibitory response is predominantly the result of a conductance increase for K^+. This differs from the situation in the frog sympathetic ganglia for which Weight & Padjen (1973*a*) find that the slow i.p.s.p. results from a decrease in Na^+ conductance.

Voltage-sensitive conductance changes during the inhibitory response

In the reversal potential curve for the inhibitory response (Fig. 9*B*) the curve becomes non-linear at membrane potentials smaller than -60 mV. To investigate this non-linearity we used two intracellular electrodes, one to record potentials and one to pass current, while inhibitory responses were evoked by either nerve stimulation or iontophoretic application of bethanechol or ACh. These experiments invariably showed that there is a bend in the response-amplitude *vs.* membrane potential curve near -60 mV. An example of this, using bethanechol as the agonist, is shown in Fig. 11. A possible source for the non-linearity is that the participation of ions other than K^+ could become increasingly significant at membrane potentials lower than -60 mV. For example, at these lower potentials the transmitter may also decrease the resting Cl^- conductance or increase the conductance for Na^+. In either event the hyperpolarizing action of K^+ would be counteracted. These possibilities were excluded by the finding that the bend in the curve was not affected when 99 % of the Na^+ in the bathing fluid was replaced with Tris or when 93 % of the Cl^- was substituted with sulphate. Another possible source, that of a conductance increase of the non-synaptic membrane, is excluded by measurements of synaptic currents presented below.

The most likely explanation for the non-linearity is that the conductance change responsible for the inhibitory potential is sensitive to membrane

voltage. To extract information about synaptic membrane currents, and therefore about conductance changes, we plotted the current–voltage curves for the neurone of Fig. 11 in the presence and absence of bethanechol (Fig. 12*A*). The potential in the presence of bethanechol is provided by the membrane potential during the peak of the inhibitory response. Attenuation of the response due to capacitance is negligible, since the

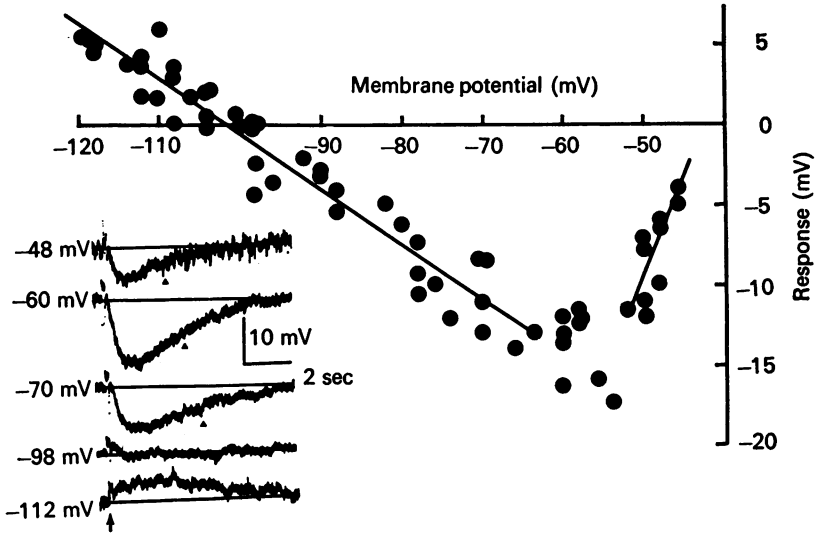

Fig. 11. Voltage sensitivity of the inhibitory response. One intracellular electrode recorded potentials and a second injected current to adjust the membrane potential. The points represent the peak responses caused by iontophoretic application of bethanechol. The curve is linear for membrane potentials more hyperpolarized than −65 mV and indicates a reversal potential of −101 mV. In the vicinity of −50 to −60 mV, however, there is a distinct bend in the curve, so that the responses become smaller as the membrane potential is depolarized. *Inset*: sample traces of the responses. The duration of the inhibitory potentials is prolonged as the cell is hyperpolarized. Arrow marks time of ACh application and triangles indicate half-times of decline. Note the spontaneous hyperpolarizations (see Fig. 2) at the membrane potential of −48 mV.

time constant of the membrane is less than 0·1 sec. The difference between the two current–voltage curves of Fig. 12*A*, ΔI, provides directly the synaptic current at the peak of the inhibitory potential. It is the same as the synaptic current that would have been obtained had the cell been voltage clamped during the inhibitory response. Thus ΔI is zero at the reversal potential (−101 mV) and increases linearly as the cell is depolarized toward −65 mV (Fig. 12*B*). However, as the cell is depolarized

further, the current becomes smaller. In several cells the membrane was depolarized as far as +20 mV, but in no case did the synaptic current drop to zero.

We conclude that the inhibitory conductance change produced by bethanechol (or by nerve stimulation) is voltage sensitive. To quantitate this voltage sensitivity we calculated the conductance change, Δg, from the equation

$$\Delta g = \Delta I/(E_m - E_{rev}),$$

where E_m is the membrane potential and E_{rev} is the reversal potential of the inhibitory response (see Takeuchi & Takeuchi, 1960). The non-linearity of ΔI as a function of E_m is assumed to be due to a voltage dependence of Δg alone. Δg was calculated using the ΔI values of Fig. 12*B*, and then plotted as a function of E_m (Fig. 12*C*, continuous line).

Fig. 12*C* also shows the half-times of decline of the inhibitory responses (circles), samples of which were illustrated in Fig. 11 inset. The responses became progressively slower as the cell was hyperpolarized to −70 mV. It is apparent that the conductance change and the time course of the responses have similar voltage sensitivities. This suggests that the rate of inactivation of the response influences the peak amplitude of the conductance change.

The delayed onset of the inhibitory response

Another consistent feature of the inhibitory response is its delayed onset or 'latency'. This is most clearly seen with iontophoretic application of bethanechol, since there is no interference from an excitatory response. Examination of the time courses of the responses to bethanechol, such as illustrated in Figs. 8 and 9, indicates that there is a latency of over 100 msec between the time of bethanechol application and the beginning of a perceptible response. The latency is better seen when the responses are examined on a fast time base and with high amplification, as in Fig. 13. The responses were signal-averaged to reduce noise, particularly that arising from spontaneous hyperpolarizations (cf. Fig. 2). Fig. 13 shows that the initial rising phase of the potentials at 14 °C is considerably delayed compared with that recorded at 24 °C. This large temperature dependence indicates that diffusion of the agonist is not a major contributor to the latency. Consistent with this conclusion is the concentration profile for bethanechol at the neuronal surface, calculated for simple diffusion (Fig. 13, shaded area). By the time any response is seen, 80 % of the bethanechol has diffused away.

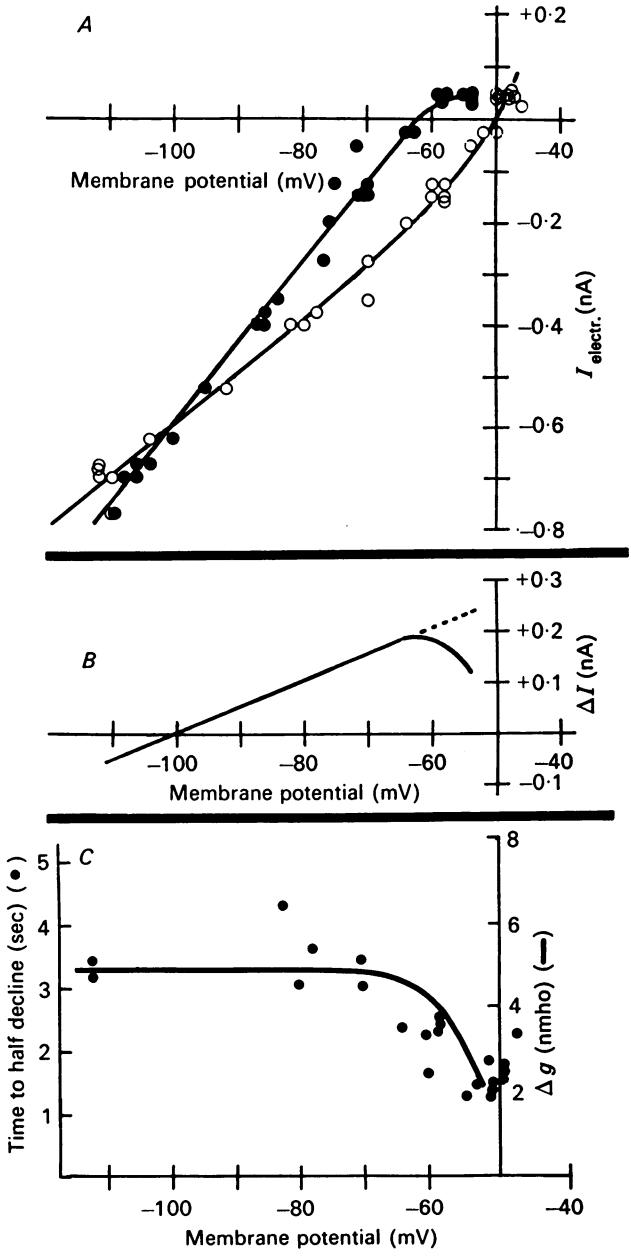

Fig. 12. For legend see opposite.

Estimates of agonist concentration at the cell surface were based on the assumption that an amount, Q, of the agonist was released from a point source as an instantaneous pulse. The concentration of agonist, [A], at a distance, x, from the source, was taken to be

$$[A] = \frac{Q}{8(\pi Dt)^{3/2}}(e^{-x^2/4Dt}) \quad (1)$$

(del Castillo & Katz, 1955), where t is time, and D is the diffusion coefficient for the agonist. The average concentration over the surface of the cell, $[\overline{A}]$, was obtained by integrating eqn. (1) and dividing by the surface area. The cell surface was approximated by a disk of radius, ρ, and agonist was assumed to be released from a distance, r, above the centre of the disk. Integration of eqn. (1) under these conditions gives

$$[\overline{A}] = \frac{Q}{2\pi^{3/2}\rho^2(Dt)^{1/2}}(e^{-r^2/4Dt})(1-e^{-\rho^2/4Dt}).$$

The curve, shown at the top of Fig. 13, is based on a value of 3 μm for r, 25 μm for ρ, and 10^{-5} cm^2/sec for D.

Similarity between inhibitory responses in neurones and cardiac muscles

In the course of our experiments we noticed that the cardiac muscles which lie among the principal cells respond to ACh and bethanechol. Both

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Fig. 12. Voltage dependence of conductance change and time course of the inhibitory response. Data from experiment of Fig. 11. *A*, current–voltage curves in the absence (open circles) and presence (filled circles) of iontophoretically applied bethanechol. The membrane potentials during the peaks of the inhibitory responses provide the potentials in the presence of bethanechol. The ordinate shows the currents passed through the electrode to vary the membrane potential (I_{electr}). Current measurements were not available for all points in Fig. 11. The lines were drawn through the points by eye. The two curves cross at the reversal potential. *B*, the difference between the two curves in *A*, ΔI, represents the synaptic current that flows during the peak of the inhibitory response. It is plotted as a function of membrane potential. The curve is linear for membrane potentials more negative than -65 mV, and the reversal of the synaptic current occurs at a membrane potential of -101 mV. At membrane potentials more depolarized than -65 mV the synaptic current is less than what would have been expected had the curve remained linear (dotted line). *C*, the conductance change, Δg, for the inhibitory response was obtained from the equation, $\Delta g = \Delta I/(E_m - E_{rev})$, taking the reversal potential, E_{rev} (-101 mV), and ΔI values from *B*. Δg is plotted (continuous line) as a function of E_m, the membrane potential. Δg is constant from -110 to about -65 mV, then declines as the cell is depolarized further. The circles indicate the half-times to decline of responses analysed in Fig. 11. No points are shown in the vicinity of -100 mV (near the reversal potential), since the responses at these membrane potentials were too small.

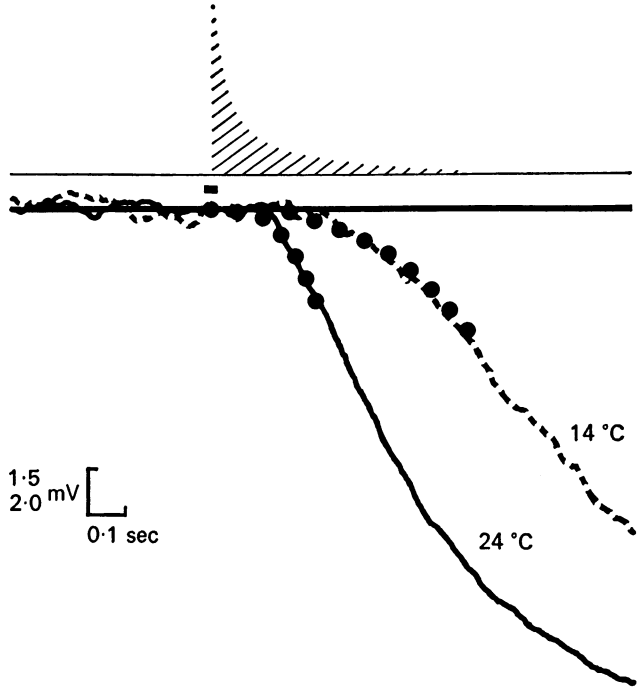

Fig. 13. Delayed onset of the inhibitory response to iontophoretically applied bethanechol. A pulse of bethanechol (200 nA for 30 msec) was released iontophoretically onto the surface of a principal cell every 30 sec. A series of nine responses was obtained at 24 °C, then the preparation was cooled and a series of 9 responses obtained at 14 °C. The preparation was returned to 24 °C and the entire process repeated. All eighteen responses at a given temperature were essentially the same and they were signal-averaged. The average peak amplitude was 20 mV at 24 °C and 15 mV at 14 °C. The bethanechol pulse is represented by the bar. Calibration: 1·5 mV, 14 °C; 2·0 mV, 24 °C. Circles (●) indicate theoretical curves, assuming that responses are proportional to t^3, where t is time. Voltage artifacts due to iontophoretic current pulses have been removed. Cooling resulted in a decrease in the resting potential of about 10 mV. The shaded curve (top) gives the estimated time course of the average bethanechol concentration at the cell surface for both temperatures, calculated from diffusion equations. For the calculation, the surface of the cell was approximated by a disk 25 μm in radius, and the bethanechol was assumed to have been applied at a distance of 3 μm from the surface (see text).

of these agents produce a pure hyperpolarizing response that can be blocked by atropine (5×10^{-9} M). We conclude that muscles of the *Necturus* heart have muscarinic receptors, as do other vertebrate hearts (Koelle, 1975*b*). By analogy we assume that activation of these receptors results in a conductance increase primarily for K^+ (Harris & Hutter,

1956; Trautwein & Dudel, 1958; Hutter, 1961). Thus, the hyperpolarizing response of the muscle resembles in many respects the inhibitory response described above for the principal cells, and therefore it was of interest to look more closely at the muscle response.

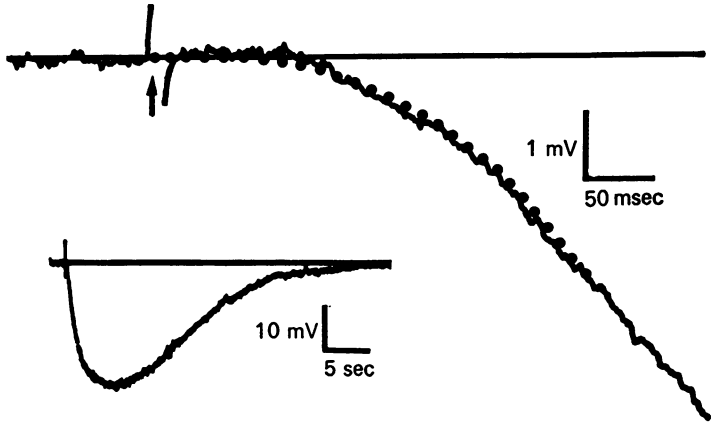

Fig. 14. Response of *Necturus* heart muscle to iontophoretically applied ACh, recorded with an intracellular electrode. A 5 msec (250 nA) pulse of ACh was applied to the surface of the muscle, and produced the response in the lower record. Ten such responses were signal-averaged, and the initial portion of the rising phase of the average response is shown in detail (upper trace). The ACh pulse (arrow) produced a voltage artifact, followed about 100 msec later by the first signs of a response. In the early phase the response is proportional to t^3 (circles, cf. Fig. 13). The temperature was 23 °C.

When a large pulse of ACh was released iontophoretically onto the muscle surface, a hyperpolarization resulted, which rose to a peak of 25 mV in 5 sec and then declined with a half-time of about 8 sec (Fig. 14, lower trace). With decreasing doses the times to peak and half-decline became progressively shorter, while the time courses of the early rising phase remained the same; there is a period of about 100 msec following the application of the agonist before one sees a clear hyperpolarization (upper trace). The initial portion of this response has kinetics similar to those of the neurone (see Fig. 13 and Discussion).

DISCUSSION

One transmitter mediates excitation and inhibition on the same neurone by activating different receptors. Our main finding is that the transmitter, ACh, liberated by vagal nerve terminals produces two distinct post-

synaptic responses in principal cells. In addition to the rapid and powerful e.p.s.p. which initiates conducted impulses (Roper, 1976*a*), nerve stimulation can produce a slow i.p.s.p. The excitatory input from the vagus is specifically blocked by the nicotinic antagonist dihydro-β-erythroidine, leaving the i.p.s.p. virtually intact. Conversely, low concentrations of atropine, a muscarinic blocker, obliterates inhibition without affecting the e.p.s.p. Furthermore, the muscarinic agonist bethanechol specifically activates the receptors that produce the i.p.s.p. Since interneurones are not involved in these responses, inhibition and excitation must result from the direct action of ACh on two types of receptors in the postsynaptic membrane of principal cells.

That one neurone should possess two types of receptors, activated by two *different* transmitters, one for excitation and one for inhibition, is a well-established rule in vertebrate nervous systems. Also established is the idea that the same transmitter can exert different actions on different cells. For example, ACh excites skeletal muscles and inhibits cardiac muscles. Furthermore, there is evidence that in some neurones of the mammalian central nervous system ACh produces fast nicotinic and slow muscarinic responses (Curtis & Ryall, 1966; Krnjević, 1975). Our study shows that in a vertebrate a single transmitter can both excite and inhibit the same neurone, as suggested by Weight & Padjen (1973*b*). Only in invertebrates, such as molluscs, has this principle been shown clearly, where ACh can produce depolarizing as well as hyperpolarizing responses in the same neurone (Wachtel & Kandel, 1971; for reviews see Gerschenfeld, 1973; Ascher & Kehoe, 1975; Kandel, 1976).

In the sympathetic ganglion of the frog the fast e.p.s.p. is known to be mediated by nicotinic receptors. However, for the slow i.p.s.p. it has yet to be resolved whether a catecholamine is involved (Tosaka, Chichibu & Libet, 1968; Libet, 1970), or whether ACh acts directly through a muscarinic receptor (Weight & Padjen, 1973*b*). For mammalian sympathetic ganglia there is evidence that dopamine causes the slow i.p.s.p. (Libet & Tosaka, 1970).

Conductance changes produced by ACh. The reversal potentials of the e.p.s.p.s in sympathetic ganglia (Nishi & Koketsu, 1960) and in the principal cells of cardiac ganglia, both in frog (Dennis *et al.* 1971) and in *Necturus*, are near -10 mV. This is similar to that of the frog neuromuscular synapse (del Castillo & Katz, 1954), where both Na^+ and K^+ conductance increases are involved (Takeuchi & Takeuchi, 1960). By analogy, we assume that the e.p.s.p. in principal cells also results from a conductance increase for these two ions. On the other hand, the reversal potential for the i.p.s.p. is close to the K^+ equilibrium level, E_K, and when $[K^+]$ in the bath is increased, the reversal potential shifts to the new E_K,

as predicted by the Nernst equation. In agreement with this finding, varying the external [Na^+] or [Cl^-] has no effect on the reversal potential. Thus, the i.p.s.p. is caused by an increase in K^+ conductance. In contrast, the inhibitory response in nicotine-treated sympathetic ganglion cells of the frog, produced by preganglionic nerve stimulation or by ACh iontophoresis, is apparently due to a decrease in Na^+ conductance (Weight & Padjen, 1973*a*), a conclusion based in part on slope conductance measurements. We could not use slope conductance measurements in parasympathetic ganglion cells of *Necturus*, since the conductance change produced during the i.p.s.p. is voltage sensitive (Fig. 12*C*).

Transmitter-evoked conductance changes that are voltage dependent have been reported for various neuromuscular synapses (Anderson & Stevens, 1973; Dudel, 1974; Anderson, Cull-Candy & Miledi, 1976). This dependence is due to a voltage sensitivity of the open-time of ionic conductance channels. Although we do not know the characteristics of the individual channels for the slow inhibitory response, it is clear from Fig. 12*C* that the time course of the overall response is voltage sensitive. The lengthening of the response with hyperpolarization could result from an increase in the open-time of individual channels or a longer time during which channels can open. Either case could contribute to the voltage sensitivity of the peak conductance change.

The time course of the inhibitory response. It is useful to compare the rapid e.p.s.p. and slow i.p.s.p. in the context of the following scheme, which presents the steps leading to the conductance changes.

$$\underset{\substack{\text{I}\\\text{diffusion}}}{\mathrm{ACh} \rightleftharpoons \mathrm{ACh} + \mathrm{R}} \underset{\substack{\text{II}\\\text{binding}}}{\rightleftharpoons \mathrm{ACh{\cdot}R}} \underset{\substack{\text{III}\\\text{activation}}}{\dashrightarrow\!\dashrightarrow\!\dashrightarrow} \begin{pmatrix} g_{\text{open}} \\ g_{\text{closed}} \end{pmatrix}$$

The interaction of ACh with receptor (R) leads *via* undefined steps (dashed arrows) to the opening of ionic conductance channels (g). For the e.p.s.p. at the neuromuscular synapse the entire process, I to III, takes place within a few milliseconds. It has been calculated that upon release from the presynaptic nerve the ACh molecules diffuse to the post-synaptic membrane within 50 μsec (Katz & Miledi, 1965). We know that the binding to receptors (step II) and activation of ionic channels (step III) are accomplished within 0·3 msec, because by that time the post-synaptic current has reached its peak (Fatt & Katz, 1952). In the reverse direction, steps III through I also occur within a few milliseconds, as measured by the declining phase of the synaptic current.

In principal cells the total duration of the e.p.s.p. is about 50 msec. It follows, then, that there is little or no free ACh left during most of the latent period of the i.p.s.p., which lasts over 100 msec. This conclusion is

supported directly by experiments in which the muscarinic receptors were activated by iontophoretic application of bethanechol. After a brief pulse of bethanechol there is a delay of at least 150 msec before any response is seen (Fig. 13). Diffusion calculations indicate that by this time the bethanechol concentration has not only peaked but has declined to about one-fifth. Thus, neither diffusion (step I) nor agonist binding (step II) can be rate limiting. Furthermore, the early portion of the potential is accurately described by response $= kt^3$, where t is time and k is the apparent rate constant for the activation of the conductance increase. The value for k for the response at 24 °C is eleven times greater than that at 14 °C. This large temperature dependence is yet another indication that diffusion of agonist (step I) is not rate limiting. We propose that step III, the activation of ionic channels by the agonist–receptor complex, is rate limiting. The third-order dependence on time for the initial phase of the response suggests that step III consists of a series of three or more reactions.

In the simplest case, the Q_{10} for the apparent rate constant, k_{app}, is the product of the Q_{10}s for the individual reactions in step III. For example, if step III consists of three reactions:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} D$$

it can be calculated that at early times, if A is assumed to remain constant, and B, C, and D are far from equilibrium (e.g. $C << [k_2B + k_{-3}D]/[k_{-2} + k_3]$), then $dA/dt = 0$, $dB/dt = k_1A$, $dC/dt = k_2B$, and $dD/dt = k_3C$. Substitutions and integrations give

$$D = k_1k_2k_3A\int\int\int dt\,dt\,dt = \frac{k_1k_2k_3A}{6}t^3,$$

and the apparent rate constant, k_{app}, is equal to $k_1k_2k_3A/6$. The Q_{10} for k_{app}, then, is given by

$$Q_{10}(\text{app}) = \frac{(k_{app})\,24\,°C}{(k_{app})\,14\,°C} = \frac{(k_1k_2k_3A/6)\,24\,°C}{(k_1k_2k_3A/6)\,14\,°C}$$

Thus, Q_{10}(app) is equal to the product of the Q_{10}s of the three individual reactions, and a Q_{10}(app) of 11, such as was found for Fig. 13, could result if each of the three individual reactions had a Q_{10} of 2·2.

In this context one should consider the possibility that some of the reactions in step III require metabolic energy, particularly in view of the proposal by Greengard (1976) and his colleagues that the genesis of slow muscarinic synaptic potentials in sympathetic ganglia may be mediated by an ACh-activated guanylate cyclase.

The inhibitory response of cardiac muscles, including that of the muscle

fibres in the vicinity of the parasympathetic principal cells that we have studied, has several features in common with the inhibitory response in principal neurones: (i) inhibition is mediated by muscarinic receptors that can be activated by ACh and bethanechol, and blocked by atropine; (ii) the inhibitory response lasts several seconds and its initial phase can be described by: response = kt^3 (Fig. 14; see also Purves, 1976); (iii) activation of muscarinic receptors leads to an increased conductance to K^+ (Harris & Hutter, 1956; Trautwein & Dudel, 1958; Hutter, 1961), and this conductance increase is voltage sensitive (Giles & Noble, 1976). These similarities suggest that the inhibitory responses produced by ACh in heart muscle and parasympathetic ganglion cells share a common underlying mechanism.

There are other examples where slow muscarinic responses have been demonstrated with latencies of several hundred msec following a brief iontophoretic application of ACh. Among these are bullfrog sympathetic ganglion cells (Koketsu, Nishi & Soeda, 1968), cultured intestinal smooth muscle cells (Purves, 1974), and smooth muscle cells from guinea-pigs (Bolton, 1976).

Physiological role of the i.p.s.p. Excitation of neurones in the parasympathetic ganglion by vagal stimulation leads to the liberation of ACh on to the cardiac muscles, thereby reducing the heart rate. Therefore, any process that reduces the excitability of ganglion cells could also reduce cardiac inhibition. It is apparent that the slow i.p.s.p. in principal cells may serve such a role. However, we do not know how effective the i.p.s.p.s are, because they were small or absent in some neurones, even though they had membrane potentials in the normal range and gave overshooting impulses upon vagal stimulation. A test for inhibition of neurones within the ganglion would be to determine whether low concentrations of atropine, which selectively block the i.p.s.p.s, increase the number of impulses emerging from the ganglion upon vagal stimulation.

Inhibitory responses that last several seconds are also of interest on general grounds. They differ markedly from the well-established rapid inhibitory and excitatory synaptic potentials with durations of a fraction of a second, which have been reported for both vertebrate and invertebrate nervous systems (Florey, 1961; Eccles, 1964). For these rapid potentials to sum, presynaptic impulses must arrive within a few milliseconds of each other. In contrast, the long duration of the slow i.p.s.p. permits temporal summation at frequencies as low as 1–5/sec. This seems well suited for a modulating action that shifts the level of neuronal excitability.

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