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PEPTIDERGIC AND MUSCARINIC EXCITATION AT AMPHIBIAN SYMPATHETIC SYNAPSES

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SUMMARY

1. A single-electrode voltage clamp was used to study the slow muscarinic and late slow peptidergic excitatory post-synaptic currents (e.p.s.c.s) in B cells of the paravertebral sympathetic ganglia of the bull-frog.

2. Conductance decreases were measured during peptidergic e.p.s.c.s in nearly all cells at clamped potentials near the resting level. In about half of the cells the size of the peptidergic e.p.s.c.s increased with hyperpolarization and in some of these cells conductance increases were found at hyperpolarized levels. In the remaining cells conductance decreases occurred at all levels of membrane potential tested, and in a few of these the polarity of the e.p.s.c.s reversed at hyperpolarized potentials. A similar diversity was observed among muscarinic e.p.s.c.s.

3. At least two simple ionic mechanisms are required to explain the heterogeneous voltage dependencies observed: a conductance decrease primarily to K^+ that dominates at depolarized potentials and a conductance increase to other ions that is more prominent at hyperpolarized potentials. The proportion of these two mechanisms appears to differ among B cells.

4. The two slow e.p.s.c.s recorded in the same neurone had the same voltage dependence and were accompanied by the same conductance changes in each of eight cells despite differences between cells. The muscarinic e.p.s.c. was reduced during the peptidergic e.p.s.c. in each of twenty-five neurones tested over a range of membrane potentials.

5. Externally-applied luteinizing hormone releasing hormone (LHRH) produced currents with the same voltage dependence and conductance changes as the nerve-evoked peptidergic e.p.s.c. in each of fifteen cells tested.

6. Bethanechol, a muscarinic agonist, and LHRH produced currents with the same voltage dependence and conductance changes in each of the twelve cells studied. In several cells a saturating response to a prolonged application of LHRH completely occluded the response to bethanechol, and vice versa.

7. Slow currents were recorded from dissociated cell bodies in response to bethanechol and LHRH; these responses exhibited the same diversity of voltage dependence and conductance changes as was observed in intact ganglia.

8. Activation of muscarinic and peptidergic receptors may control shared ionic mechanisms in single ganglion cells.

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INTRODUCTION

The excitatory signals at skeletal nerve–muscle synapses in vertebrates and inhibitory signals at crustacean nerve–muscle synapses are relatively rapid, lasting for only a small fraction of a second (e.g. Katz, 1969; Takeuchi, 1978). Fast synaptic potentials with similar ionic mechanisms have been found at many synapses in the central nervous system of all the vertebrates that have been examined (e.g. Eccles, 1964; Kuno, 1971; Bennett, Model & Highstein, 1976; Llinas & Nicholson, 1976). In contrast to these fast responses, some neurones in the autonomic and central nervous systems produce electrical responses to chemical signals which last for many seconds or minutes (Laport & Lorente de No, 1950; Eccles, 1952; Eccles & Libet, 1961; Krnjević, 1974).

Attention has recently been focussed on the paravertebral sympathetic ganglia of the frog in which four distinct synaptic signals are produced by nerve stimulation (Skok, 1973; Nishi, 1974; Volle, 1975; Ginsborg, 1976; Kuba & Koketsu, 1978; Libet, 1979; Kuffler, 1980; Weight, 1982). Following stimulation of preganglionic fibres, release of acetylcholine (ACh) from nerve terminals evokes in a ganglion cell a fast excitatory post-synaptic potential (e.p.s.p.) lasting tens of milliseconds, usually initiating an impulse. The release of ACh also produces two slow potentials: a slow inhibitory post-synaptic potential lasting for several seconds in a class of small ganglion cells innervated by slowly conducting axons (C cells) and a slow e.p.s.p. lasting up to 1 min in a separate class of large ganglion cells innervated by rapidly conducting axons (B cells). In addition to the three responses mediated by ACh, a non-cholinergic slow depolarizing response called the late slow e.p.s.p., lasting up to 10 min, can be produced in most ganglion cells by a train of impulses in the 7th and 8th nerves, as first described by Nishi & Koketsu (1968). Recent evidence indicates that the transmitter for the late slow e.p.s.p. is a peptide resembling luteinizing hormone releasing hormone (LHRH) (Jan, Jan & Kuffler, 1979, 1980; Kuffler, 1980; Jan & Jan, 1982).

We report here the use of a voltage-clamp procedure to measure the slow synaptic currents directly. Unlike the fast e.p.s.p. in these cells, and unlike the slow synaptic potentials studied in other preparations (Hartzell, Kuffler, Stickgold & Yoshikami, 1977; Kehoe & Marty, 1980; Hartzell, 1981), the voltage dependence of the slow e.p.s.c.s, both muscarinic and peptidergic, differed significantly among neurones that otherwise could not be distinguished, in agreement with current-clamp measurements of the slow e.p.s.p.s (Kuba & Koketsu, 1976; Nishi & Katayama, 1981). This diversity of properties required a more complex explanation than a simple decrease in K^+ conductance which had previously been suggested as the underlying mechanism (Weight & Votava, 1970; Adams & Brown, 1980; Adams, Brown & Constanti, 1982).

The fast nicotinic e.p.s.p. is enhanced during the slow peptidergic e.p.s.p. (Schulman & Weight, 1976), but the slow muscarinic e.p.s.p., surprisingly, is reduced (Jan *et al.* 1979; Jan & Jan, 1982); this suggests that the slow excitatory currents are produced, at least in part, by a shared mechanism. Bath-applied muscarine and LHRH both inactivate a voltage-sensitive K^+ current in these cells (Brown & Adams, 1980), further strengthening this possibility. In view of the heterogeneity in the voltage dependence of the muscarinic and peptidergic responses among ganglion cells

and the likelihood that several mechanisms contribute, we have investigated the interaction of both nerve-evoked and agonist-induced responses in individual cells at different levels of membrane potential.

Preliminary reports of this work were presented previously (Kuffler, 1980; Sejnowski & Kuffler, 1981; Sejnowski, 1982).

METHODS

Preparation

All experiments were performed on B neurones in the 9th and 10th paravertebral sympathetic ganglia of the bull-frog (*Rana catesbeiana*). The last four ganglia of the paravertebral chain, designated 7–10 (Ecker & Wiedersheim, 1896; Pick, 1957), were isolated together with the spinal nerves which innervate them. The ganglia were cleaned of connective tissue, pinned out in a small chamber, and prepared for recording as described by McMahan & Kuffler (1971). The bathing solution had a volume of less than 0·5 ml and was continuously exchanged at a rate of 0·4–10 ml./min.

Individual neurones in the thinner portions or around the edges of the ganglion were visualized under Nomarski differential-interference-contrast optics and impaled with micropipettes. The ganglion cells are monopolar; synapses from the preganglionic cholinergic fibres are densely distributed over the soma and axon hillock (Weitsen & Weight, 1977; Marshall, 1981; Jan & Jan, 1982). The B cells that were studied averaged aroung 50 μm in diameter; resting potentials after impalement were usually between -40 mV and -70 mV, and input resistances were 20–100 MΩ. Two other types of cells in these ganglia, the smaller C cells (Horn & Dodd, 1981; Dodd & Horn, 1983) and a sparse population of s.i.f. (small-intensely-fluorescent) cells containing catecholamines (Weight & Weitsen, 1977; Watanabe, 1980), were not examined.

Nerves were stimulated with suction electrodes. The slow muscarinic e.p.s.p. could be produced in B cells by stimulating the preganglionic chain above the 7th ganglion while the slow peptidergic response could be evoked by stimulating the 7th and 8th roots emerging from the spinal cord (Nishi, Soeda & Koketsu, 1965; Libet, Chichibu & Tosaka, 1968; Nishi & Koketsu, 1968). In some experiments antidromic action potentials were elicited in ganglion cells by stimulating the post-ganglionic nerves.

Solutions

The Ringer solution normally contained (mM): NaCl, 115; $CaCl_2$, 3·6 and KCl, 2·0; buffered at pH 7·3 with 1 mM-Na HEPES (Na-*N*-2-hydroxyethylpiperazine-*N'*-ethane sulphonate). In some experiments the concentration of KCl was increased up to 6 mM without altering the other components. Nicotinic transmission was sometimes blocked by 1 μM-dihydro-β-erythroidine (gift of Merck Sharp & Dohme) and muscarinic transmission by 1 μM-atropine sulphate (Merck). Prostigmine bromide (1–3 μM) was used in some experiments to block cholinesterase. All experiments were performed at room temperature, 22–26 °C.

Agonist application

For some experiments, synthetic LHRH (Calbiochem) or carbamyl-β-methylcholine chloride (bethanechol, Sigma) was dissolved in Ringer solution and was delivered to individual cells in a capillary pipette having an inner diameter of 2–5 μm at its tip. The pipette was positioned 10–50 μm from the target cell body and pointed against the flow of the perfusion fluid, as shown in Pl. 1. A fluid stream from the pipette could be directed at the cell by applying 2–20 lbf/in^2 pressure to the pipette. The concentration of the drug in the vicinity of the cell body depended on many factors, such as thickness of the connective tissue surrounding the cell and the duration of the pressure application; thus, the drug concentration in the pipette represents only an upper limit for the concentration that could have been reached at the surface of the cell.

Cell dissociation and culture

The 9th and 10th paravertebral ganglia were dissected from the animal, de-sheathed and minced with forceps into small pieces. These fragments were incubated at 37 °C for 30 min in 5 ml. of enzyme solution with gentle stirring. The enzyme solution consisted of Ringer solution without Ca^{2+} containing 1 mg/ml. collagenase (Worthington 4196 CLS, 200 u/mg) and 5 mg/ml. dispase (Boehringer Mannheim 165–859, 0·5 u/mg). The cells were triturated fifty times with a fire-polished

Pasteur pipette and incubated for an additional 30 min in enzyme solution without collagenase. The cells were then centrifuged and washed three times in Ringer solution. Sterile techniques were used throughout.

For long-term culture, the cells were kept at 14 °C in plastic tubes containing Ringer and 0·1 mg/ml. bovine serum albumin, 5 mM-glucose, and 1 mM-glutamine. The cells appeared spherical and some had a small mound which may have been the former axon hillock region. The cells remained in suspension and could be drawn off with a pipette and resuspended in the recording dish. They were immobilized during recording between the tip of a drawn fire-polished capillary tube and a layer of silicone elastomer (Sylgard: Dow-Corning) on the bottom of the dish.

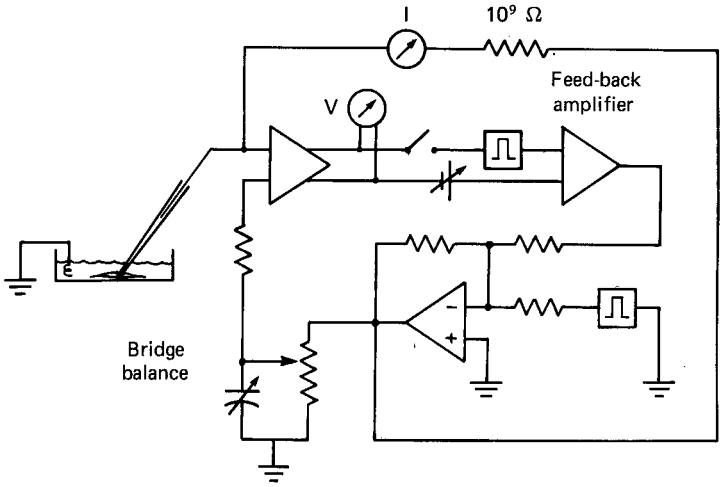

Fig. 1. Schematic drawing of the single-electrode voltage-clamp circuit. Before impaling a cell the bridge balance was adjusted to compensate for the electrode resistance. A bridge capacitor (generally 0·1 μF or less) was required to prevent oscillations when the cell was clamped through the high-gain feed-back amplifier (Tektronix AM-501 modified for a variable gain of $1–10^4$).

Electrical recording

Glass micro-electrodes were pulled on a Brown-Flaming puller from thin-wall capillary tubing having an outer diameter of 1·0 mm or 1·2 mm and a wall thickness of 0·15 mm. The micropipettes were filled with 3 M-KCl and had resistances of 20–30 MΩ. Current was passed into a cell through the recording micro-electrode using a conventional Wheatstone-bridge circuit having a 1000 MΩ resistor in series with the micropipette (Fig. 1). The membrane potential of a cell was clamped by feeding back the output from the recording amplifier to the bridge circuit through a high-gain amplifier in order to inject current into the cell.

This procedure may introduce errors if the resistance of the recording electrode, balanced by the bridge before entering the cell, changes inside the cell. Consequently, only those experiments in which the micro-electrode resistance did not change by more than 5 MΩ following withdrawal from the cell were considered valid. Voltage- and time-dependent changes in electrode resistance were not a problem because relatively low-resistance micro-electrodes and relatively small currents were used. In a few experiments the cell was impaled with two micro-electrodes in order to verify the effectiveness of the voltage control (Fig. 2).

The limiting factor in studying the slow responses was stability; only cells whose resting potential did not vary by more than 5 mV during the period of observation were accepted. From 2 to 5 min was allowed between muscarinic e.p.s.c.s to allow for recovery, and 10 to 20 min was allowed between peptidergic e.p.s.c.s. Intracellular voltages and currents were recorded continuously on a Brush pen recorder and on magnetic tape using a Hewlett Packard 3968A FM tape recorder. The data were analysed later with a Nicolet 2909 digital oscilloscope.

Voltage-jump procedure. During many responses the voltage was stepped between two values every 2–5 sec. This procedure allowed slow current responses to be measured concurrently at two clamped potentials, as shown in Fig. 2. The voltage jump also allowed the membrane conductance to be measured during the synaptic response because the 'instantaneous' jump in the current required to make a fixed voltage jump is proportional to the membrane conductance. The current jump is followed by a slower relaxing current representing a voltage-dependent conductance change (Brown & Adams, 1980; Adams *et al.* 1982), as shown in Fig. 2. As a consequence, the change in the slope conductance, as determined by the change between steady-state current envelopes at two nearby membrane potentials, cannot be used to estimate synaptic conductance changes. However, at hyperpolarized levels the current relaxations are absent, and the envelope of the steady-state current is coincident with the instantaneous current jumps.

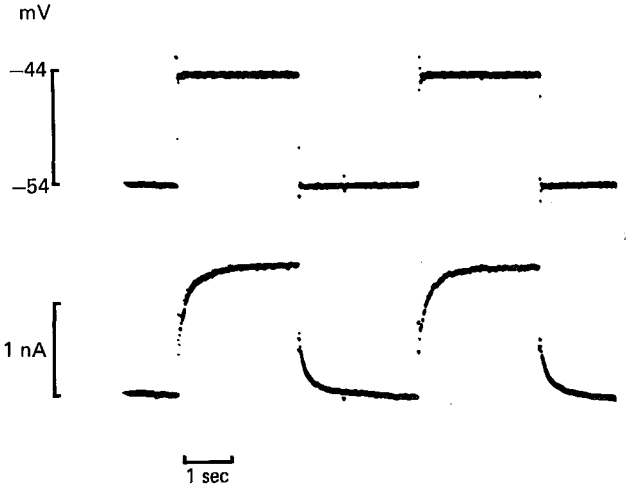

Fig. 2. Current injected into a ganglion cell (bottom) using a single-electrode voltage clamp while a second electrode in the cell passively recorded the membrane potential (top). The command voltage was stepped between −42 and −52 mV every 5 sec. The 'instantaneous' current jump (after the initial capacitative current transient settled down in less than 10 msec) was 0·45 nA from −54 to −44 mV and 0·72 nA from −44 to −54 mV. The fast current jump was followed by a slower relaxation of the current to a new steady-state level.

RESULTS

Nerve-evoked responses

The slow peptidergic response

When the 7th and 8th spinal nerves emerging from the spinal cord were stimulated with a train of 20–100 stimuli at a rate of 5–10/sec^{-1}, a very slow depolarization was recorded with an intracellular electrode from nearly all neurones in the 9th and 10th ganglia. The depolarization usually reached a peak amplitude of 5–10 mV in about 1 min, decayed in 5–10 min, and was unaffected by cholinergic antagonists. This non-cholinergic response was mimicked by synthetic LHRH and was blocked by analogues of LHRH (Jan, Jan & Kuffler, 1979, 1980; Jan & Jan, 1982). In the large B cells of the 9th and 10th ganglion no other response was elicited by stimulating the 7th and 8th spinal nerves, although in the same cells the fast nicotinic and the slow muscarinic e.p.s.p.s were produced by stimulating the chain above the 7th

ganglion. Each of the three e.p.s.p.s in such a B cell could be independently blocked with a pharmacological agent.

In all neurones that were voltage clamped at their resting potential, nerve stimulation produced a slow inward e.p.s.c. with approximately the same time course as the e.p.s.p. The slowness of the rising phase made the latency of the response difficult to judge, although a definite change above base-line noise could be discerned 1–2 sec after the start of the stimulation.

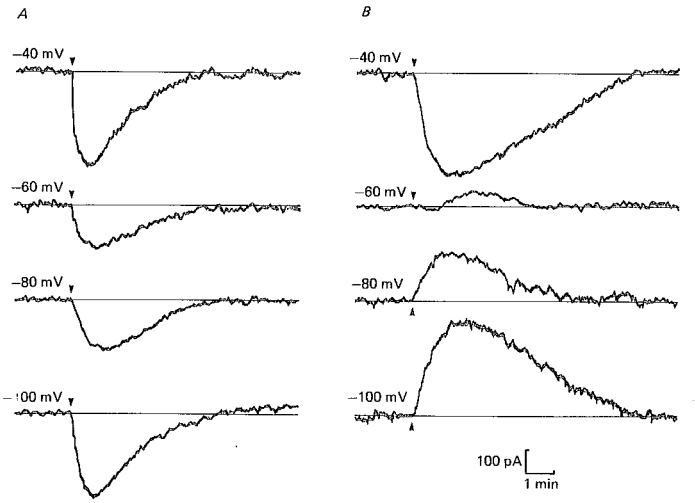

Fig. 3. Nerve-evoked peptidergic e.p.s.c.s in two representative sympathetic neurones: *A*, a 'non-reversing' type of response and *B*, a 'reversing' type of response. The cells were voltage clamped at the membrane potential indicated to the left of each current recording and the 7th and 8th spinal nerves carrying peptidergic fibres were stimulated at 20 Hz for 5 sec (arrowheads). The resting potentials of both cells were around −50 mV and the concentration of K^+ in the bathing solution was 2 mM.

Voltage dependence. The peak of the peptidergic e.p.s.c. varied non-linearly with clamped membrane potential in all cells studied; moreover, the form of the non-linearity differed among cells. The synaptic current remained inward in sixteen of twenty-two cells that were clamped at membrane potentials between −30 mV and −120 mV. In the other six cells the polarity of the synaptic current reversed beyond a hyperpolarized potential. However, in none of these cells was it possible to find a potential at which the response was null for its entire duration. The 'reversing' cell in Fig. 3*B*, for example, gave a null response for the first minute while clamped at −60 mV, but for the later portion of the response the current was already reversed in polarity. This multiphasic behaviour was particularly prominent in a few cells where it was possible to reverse the early but not the later part of the e.p.s.c. (Fig. 4).

The diversity of voltage dependence among peptidergic responses could not be related to any other distinguishing property of the cells, such as their size, input resistance, resting potential or action potential. In a few cells the voltage dependence changed in the course of a long penetration. For example, the synaptic current of

one carefully-studied cell could not be reversed during the first hour, but consistently reversed in polarity during the second hour.

Conductance changes. A 10–20 % decrease in the membrane conductance almost always accompanied the peptidergic response when a cell was clamped near, or inside-positive, with respect to its resting potential; however, in a few cells conductance decreases of 80 % or more were recorded, corresponding to a more than four-fold increase of the input resistance.

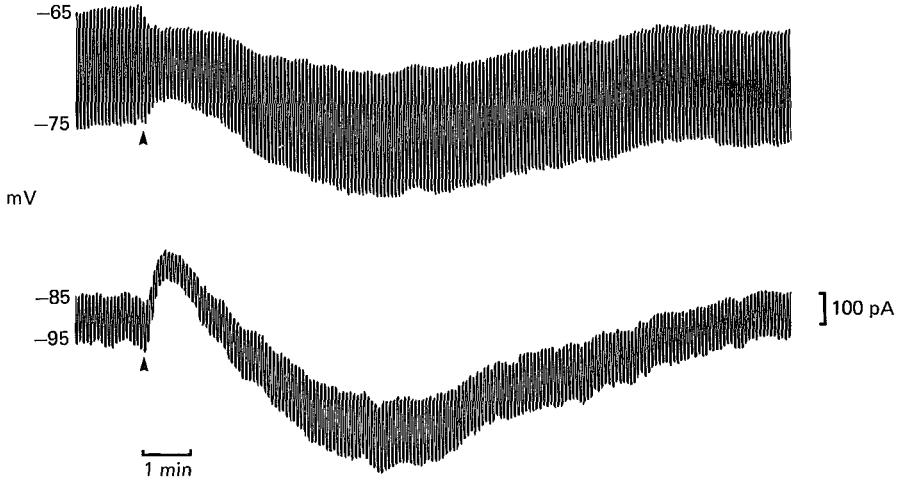

Fig. 4. Current responses in a voltage-clamped ganglion cell following stimulation of the 7th and 8th spinal nerves at 10 Hz for 5 sec (arrowheads). The membrane potential was stepped between the voltages shown to the left of each trace. The resting potential of the cell was −60 mV. Dihydro-β-erythroidine and atropine were present in the bathing solution and the concentration of K^+ was 2 mM.

Conductance increases were observed at hyperpolarized potentials in thirteen out of sixteen non-reversing cells, such as the cell in Fig. 3*A*. In contrast, only conductance decreases were observed at all membrane potentials in reversing cells. In a few cells it was possible to record both a conductance decrease and a conductance increase at the same membrane potential. For the cell illustrated in Fig. 4, a 45 % conductance decrease occurred during the first minute of the response at −85 mV and was associated with a reversal of the polarity, whereas a conductance increase of 30 % occurred during the later portion of the response that did not reverse.

The slow muscarinic response

Each ganglion cell is innervated by at least one and often several cholinergic preganglionic nerve fibres. When the chain above the 7th ganglion was excited by a short train of stimuli, in most B cells a slowly rising depolarization followed the fast nicotinic e.p.s.p.s, reached a peak in about 5 sec and decayed in 30–60 sec. The slow response was unaffected by dihydro-β-erythroidine, but could be blocked completely by atropine. Thus, ACh released from preganglionic fibres activated two distinct types of receptors on ganglion cells, nicotinic receptors and muscarinic receptors.

The slow muscarinic e.p.s.p. was sometimes evoked by a single stimulus, and increased in amplitude with the number of stimuli. When the membrane potential of a cell was voltage clamped at the resting potential, the shape of the e.p.s.c. was approximately a mirror image of the e.p.s.p. (Fig. 5). If prostigmine, which inhibits the hydrolysis of ACh, was added to the perfusate, the time to peak increased from 5 sec to about 10 sec in most neurones and the amplitude of the slow muscarinic e.p.s.p. was enhanced (Fig. 5). The nicotinic response was also significantly prolonged,

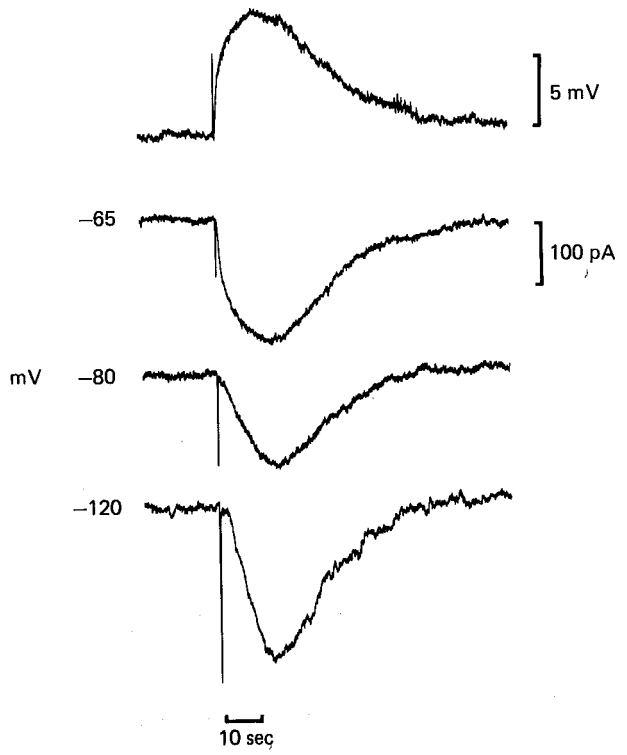

Fig. 5. Slow muscarinic e.p.s.p. (top) following five stimuli at 50 Hz to the sympathetic chain. Below are three e.p.s.c.s with the membrane potential of the cell clamped to the voltage shown to the left of each current trace. The resting potential of the cell was −67 mV. Dihydro-β-erythroidine and prostigmine were present in the bathing solution and the concentration of K^+ was 4 mM.

sometimes lasting more than 1 sec, but this component could be blocked by dihydro-β-erythroidine.

Voltage dependence. The polarity of the muscarinic response reversed at hyperpolarized potentials in nine of eighteen cells studied in the absence of prostigmine. The synaptic current of the other nine cells could not be reversed at any membrane potential tested between −30 and −120 mV. The same variability was observed when prostigmine was used to enhance the response, with reversals of polarity occurring in nine of twenty-three cells studied. In the majority of the cells the synaptic current increased, the apparent latency increased, and the rate of rise of the synaptic current decreased with hyperpolarization, as shown in Fig. 5. In some cells

the early portion of the e.p.s.c. reversed in polarity but the later portion remained inward (Fig. 6).

Conductance changes. Conductance decreases of 10–15 % usually occurred during the muscarinic e.p.s.c. when cells were clamped at membrane potentials near their resting potentials or at depolarized levels. Conductance increases occurred during muscarinic e.p.s.c.s at hyperpolarized potentials in nine cells, all of which were non-reversing. In reversing cells only conductance decreases were measured at all membrane potentials tested.

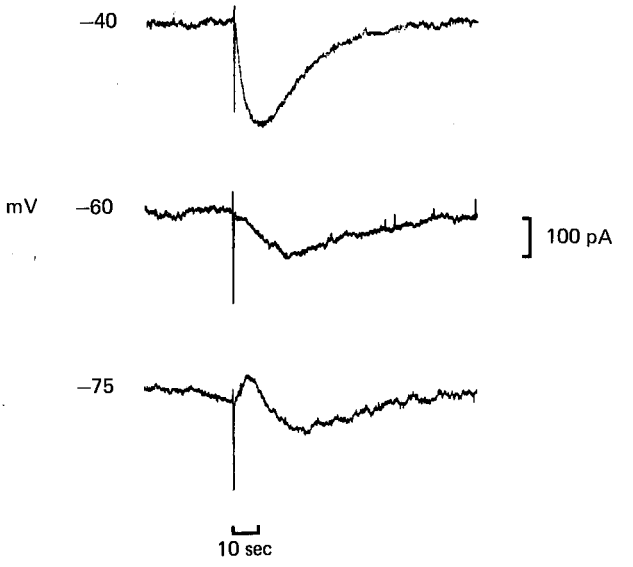

Fig. 6. Slow muscarinic e.p.s.c.s following six stimuli at 50 Hz to the sympathetic chain. The clamped membrane potential is indicated to the left of each trace. The resting potential of the cell was −35 mV. Dihydro-β-erythroidine and prostigmine were present in the bathing solution and the concentration of K^+ was 6 mM.

Peptidergic and muscarinic e.p.s.c.s in the same cell

Muscarinic and peptidergic e.p.s.c.s were recorded from the same cells over a wide range of membrane potentials. In four of eight neurones the two synaptic currents increased with hyperpolarization and were accompanied by similar conductance changes. For the cell in Fig. 7 there was a decrease in the instantaneous conductance of 25 % at the peak of the muscarinic response as determined with voltage jumps from −60 to −70 mV; under the same conditions, the instantaneous conductance of the cell at the peak of the peptidergic response decreased by 32 %. There was an increase in conductance of up to 27 % during the muscarinic response and up to 35 % during the peptidergic response when the voltage jumps were between −110 mV and −120 mV. The conductance increases occurred later and lasted longer at −110 mV to −120 mV compared to the time course of the conductance decreases measured at −60 mV to −70 mV.

In one cell the muscarinic and peptidergic e.p.s.c.s were biphasic at hyperpolarized

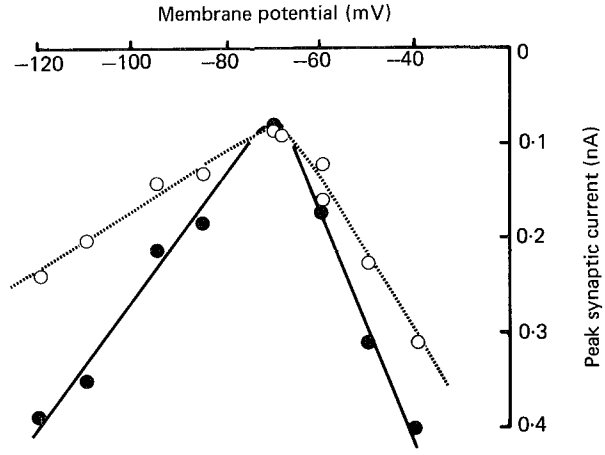

Fig. 7. Peak synaptic current in a single ganglion cell plotted against the voltage-clamped membrane potential for both muscarinic responses (○) and peptidergic responses (●). A train of 25 stimuli of the chain above the 7th ganglion at 50/sec was delivered to produce a muscarinic response and was alternated with a train of 100 stimuli of the 7th and 8th spinal nerves at 20/sec to produce a peptidergic response. The continuous and dotted lines were drawn by eye.

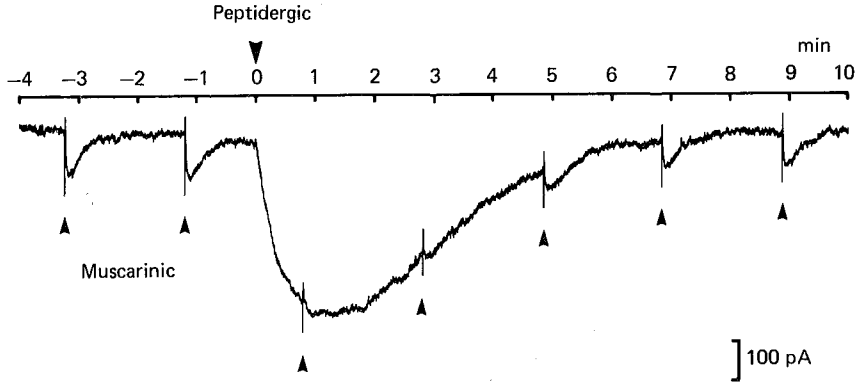

Fig. 8. Interaction between muscarinic and peptidergic e.p.s.c.s. The neurone was voltage clamped at its resting potential, −58 mV, and the cholinergic input was stimulated ten times at 50/sec repeated every 2 min. The fast nicotinic responses are cut off (arrowheads). The size of the muscarinic current was reduced following a train of 100 stimuli at 20/sec to the 7th and 8th nerves and recovered with the decline of the peptidergic current.

potentials; however, the early part of the muscarinic e.p.s.c. reversed at a less hyperpolarized potential than the early part of the peptidergic e.p.s.c. In the other three of eight cells the amplitude of the muscarinic and peptidergic e.p.s.c.s decreased with hyperpolarization until they changed polarity at the same reversal potential. Only conductance decreases were measured in these cells at all membrane potentials tested.

Interaction between the peptidergic and muscarinic e.p.s.c.s

An example of a typical interaction experiment is shown in Fig. 8. A ganglion cell was voltage clamped at its resting potential; a standard train of ten stimuli, applied to the chain at a rate of 50/sec, produced a slow muscarinic e.p.s.c. of peak amplitude 100 pA that could be reliably reproduced every 2 min. Midway between two muscarinic responses, a peptidergic e.p.s.c. was initiated by stimulating the 7th and 8th nerves for 5 sec at a rate of 20/sec. In 1 min the peptidergic response had reached a peak of about 500 pA, at which time a standard stimulation of the muscarinic response produced an additional current of no more than 10 pA, or 10 % of its previous control value. As the peptidergic e.p.s.c. decayed over the following 10 min, the additional current evoked by the standard muscarinic stimulation, repeated every 2 min, gradually recovered to within 90 % of its control value.

In twenty-five responses from twelve cells at membrane potentials between −30 mV and −120 mV the slow muscarinic e.p.s.c. was reduced by an average of 57 % at the peak of the slow peptidergic e.p.s.c. compared with control responses before and after the peptidergic response. The size of the reduction varied considerably among cells, from less than 10 % to more than 95 %, and in the same cell varied with membrane potential. In one cell a reduction of less than 10 % occurred at −50 mV, but at −40 mV the current was depressed by 60 %; however, in other cells the reduction increased with hyperpolarization. The variability of the reduction was not correlated with the amplitude of the peptidergic e.p.s.c.

Agonist-induced responses

The study of the slow peptidergic e.p.s.p. was aided by external application of LHRH in place of nerve stimulation and by block of the response with LHRH analogues that are potent antagonists (Rivier & Vale, 1978; Jan *et al.* 1979, 1980; Jan & Jan, 1982). Responses to externally applied LHRH were examined in forty-four ganglion cells. Conductance decreases occurred in all cells at their resting potential and conductance increases were detected in twenty-six cells at hyperpolarized potentials. The response to LHRH increases with hyperpolarization in twenty-four cells out of thirty-five cells examined over a complete range of membrane potentials. In the remaining eleven cells the response decreased with hyperpolarization and in nine of these cells a part or all of the LHRH-induced current reversed polarity from an inward to an outward current.

Comparison of LHRH-induced and nerve-evoked peptidergic responses

The responses evoked by LHRH depended greatly on its ease of access to the neurone and the duration of application; for example, the responses were smaller and longer when connective tissue layers covered the neurones, while in some well-exposed neurones the LHRH responses were briefer than the nerve-evoked synaptic potentials in the same neurones. Despite the quantitative differences, the LHRH-induced and nerve-evoked peptidergic responses had the same qualitative voltage dependence and were accompanied by the same conductance changes in each of fifteen neurones tested.

The neurone in Fig. 9, for example, responded with the same sequence of conductance changes, an initial conductance decrease followed by an increased

conductance at the peak of the response, to both nerve stimulation and the LHRH application. There was also an increase in excitability during the peak of both responses, as judged by the presence of regenerative currents following depolarizing voltage jumps.

The size of the conductance changes depended on the number of nerve stimuli and on the concentration of applied LHRH. In a few cells it was necessary to increase the stimulation before a qualitative similarity between the nerve-evoked and LHRH responses was apparent.

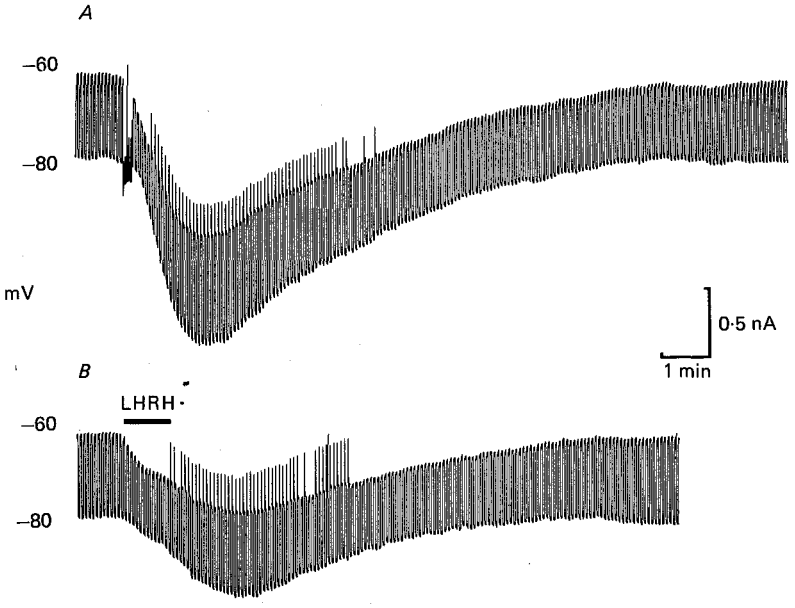

Fig. 9. *A*, peptidergic e.p.s.c. following 100 stimuli at 10 Hz of the 7th and 8th spinal nerves and *B*, peptidergic response to a 1 min application of 10^{-4} M-LHRH in the same neurone. The clamped membrane potential was alternately stepped between −60 mV and −80 mV every 5 sec. The bathing solution contained dihydro-β-erythroidine and atropine..

Comparison of bethanechol and LHRH responses in the same cell

The responses of twelve cells to applied bethanechol and LHRH were studied over a range of membrane potentials. In four of these cells the responses to both agonists reversed at the same membrane potential; in three of these cells the responses to both agonists were biphasic at the same membrane potential and in the remaining five cells neither response reversed at any membrane potential tested.

An example of a cell whose agonist-induced responses were reversed is shown in Fig. 10. The amplitudes of the responses to bethanechol and LHRH were matched in this cell by varying the duration of the pressure application; both responses lasted for 2–3 min. Under voltage clamp, the additional current across the membrane of the cell after 10 sec in response to external application of bethanechol or LHRH is plotted as a function of membrane potential in Fig. 10*B*. Reversals of these early currents evoked by both agonists occurred between −60 and −70 mV. However, no membrane potential was found at which the current was zero for the entire duration of the

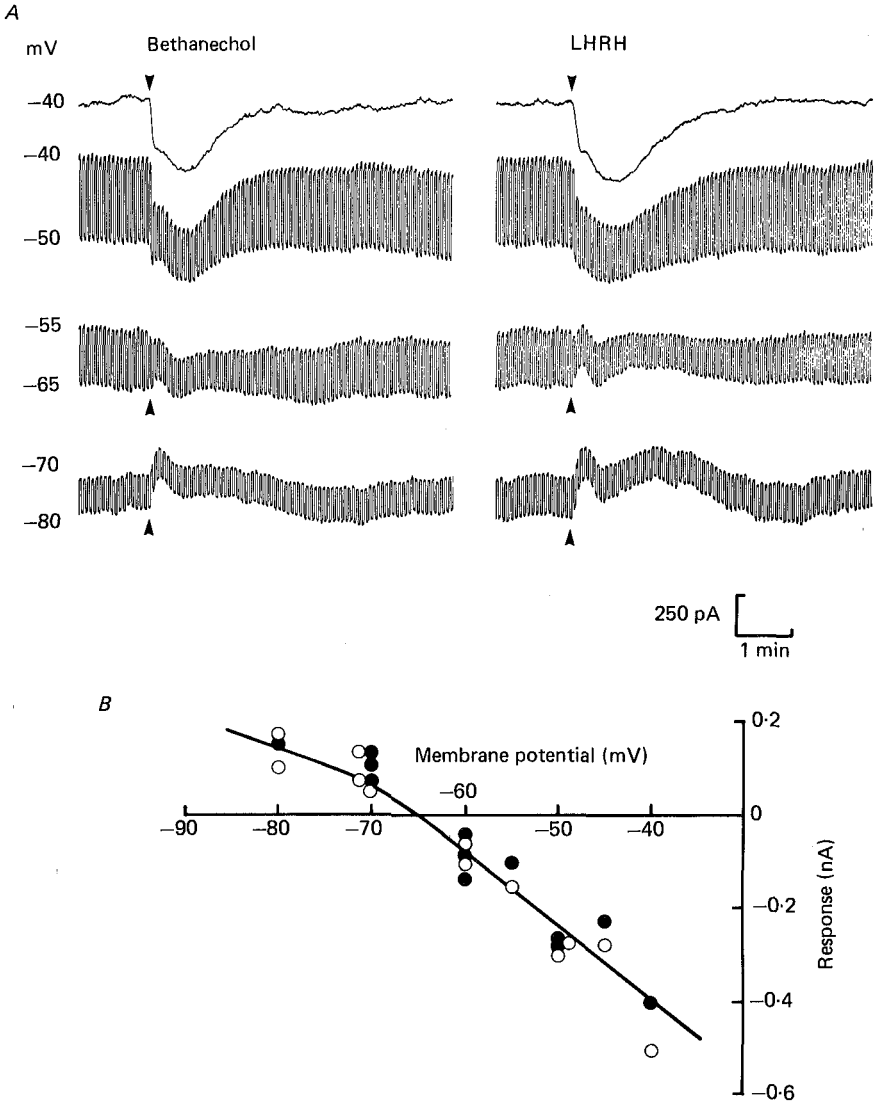

Fig. 10. *A*, voltage-clamp current recordings during the slow responses to either bethanechol or LHRH in the same synpathetic ganglion cell. The responses on the left are to a 2 sec application of 10^{-3} M-bethanechol and the responses on the right are to a 5 sec application of 10^{-4} M-LHRH. In the top pair of current recordings the neurone was clamped to −40 mV and in the recordings beneath these the membrane potential was alternately clamped between −40 mV and −50 mV every 4 sec. The envelope of the steady-state currents following voltage steps to −40 mV matches closely the continuous current response at −40 mV. The responses are also shown while the membrane potential was stepped between −55 mV and −65 mV, and between −70 mV and −80 mV. *B*, the change in current 10 sec after the onset of agonist application is plotted against the voltage-clamped membrane potentials for responses to bethanechol (○) and LHRH (●). The continuous line was drawn by eye.

response. Despite the complex shapes of the responses to bethanechol and LHRH, the details of the two responses were similar at all membrane potentials tested.

Interaction between responses to bethanechol and LHRH

Prolonged application of either bethanechol or LHRH to a cell clamped at its resting potential produced an inward current that reached a steady state in about 2 min (Fig. 11). No sign of desensitization was apparent after applications lasting up

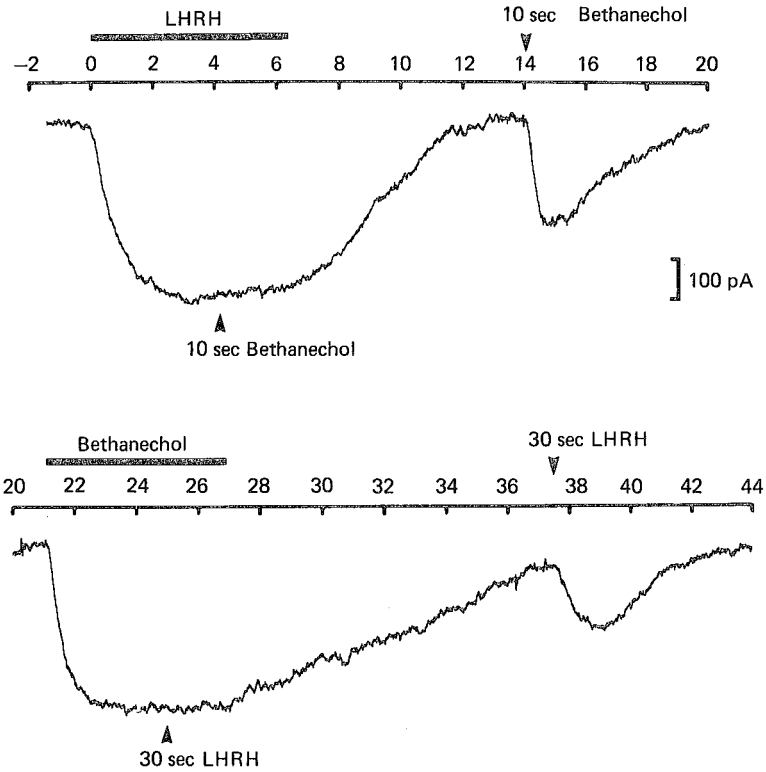

Fig. 11. Interaction between the responses to applied bethanechol and LHRH. A continuous 45 min current recording is shown during which a ganglion cell was voltage clamped at its resting potential, −59 mV. Pressure was applied to pipettes containing either 10^{-3} M-bethanechol or 10^{-4} M-LHRH (cf. Pl. 1) for varying durations, as indicated.

to 10 min. For well-exposed cells the amplitude of the inward current approached maximum with 10^{-3} M-bethanechol or 10^{-4} M-LHRH in the pipette.

In Fig. 11, a continuous 45 min current record is shown for a cell whose membrane potential was voltage clamped at its resting potential, −59 mV, while 10^{-3}M bethanechol and 10^{-4}M-LHRH were applied for varying durations. A brief 10 sec application of bethanechol during the steady-state response to LHRH produced no measurable change in the current; however, a similar 10 sec application of bethanechol after the LHRH application produced peak inward currents of more than 250 pA. Conversely, a 30 sec application of LHRH during the steady-state response to bethanechol was ineffective, but a similar 30 sec application of LHRH after the

bethanechol application produced a peak inward current of more than 175 pA. In this cell the responses to either bethanechol or LHRH could be reversed in polarity at hyperpolarized potentials; mutual blocking between the responses also occurred when the cell was voltage clamped at −100 mV, where the responses were outward currents. Complete mutual blocking of the responses to bethanechol and LHRH were also observed in cells whose muscarinic and peptidergic responses could not be reversed in polarity. In a control experiment where one of the pipettes was replaced by Ringer solution, the peak response of the cell to the agonist was reduced by less than 10% during a prolonged pressure application of Ringer.

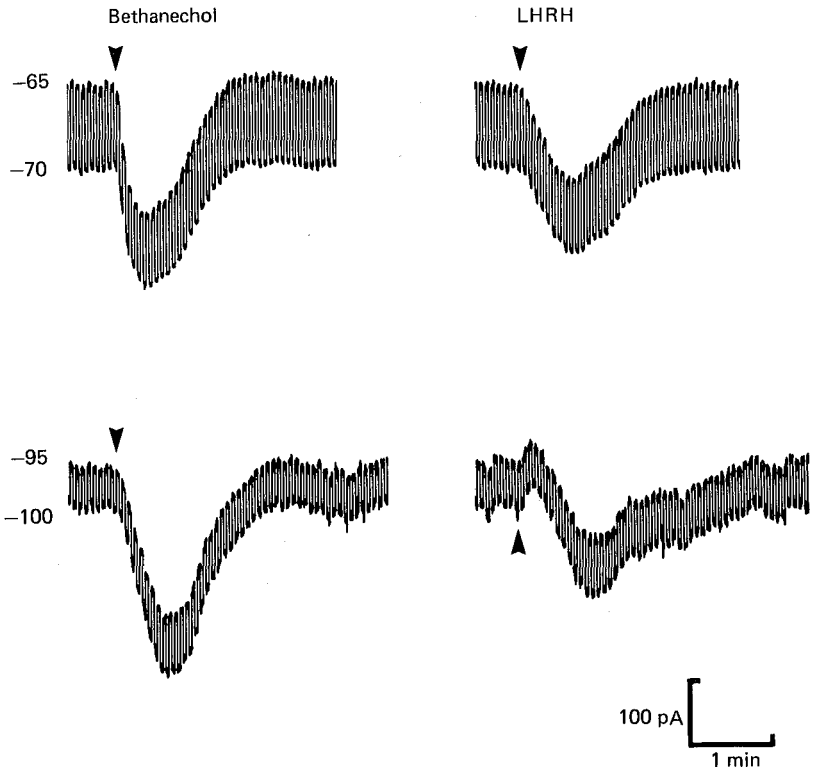

Fig. 12. Current responses of a solitary cell body dissociated from a ganglion the previous day to application of 1 mM-bethanechol (left arrows) or 10^{-4} M-LHRH (right arrows). The clamped membrane potential was alternately stepped between the voltages indicated to the left of the records.

Responses in dissociated ganglion cells

Dissociated ganglion cells cultured up to 21 days had normal action potentials to injected current pulses. In most large cell bodies the application of bethanechol and LHRH produced slow, depolarizing responses lasting several minutes. The voltage dependence of the agonist-induced current responses and the accompanying conductance changes were similar to those in intact ganglion cells.

In eighteen of twenty-two dissociated neurones the voltage dependence of the responses to bethanechol and LHRH were the same: both responses increased with

hyperpolarization in five cells and decreased with hyperpolarization in thirteen cells, with reversals in the polarity of the current occurring at the same membrane potentials in each of nine cells. In the other four of twenty-two dissociated neurones the responses to LHRH reversed but the response to bethanechol either did not reverse or disappeared with hyperpolarization. The cell of Fig. 12, for example, had a response to LHRH that reversed in polarity during the first 30 sec at −95 mV in three consecutive trials, but the response to bethanechol at the same membrane potential had no reversal. Nevertheless, conductance decreases occurred during both responses at −70 mV and conductance increases occurred during the peaks of both responses at −100 mV.

DISCUSSION

Comparison of the slow e.p.s.p.s and fast e.p.s.p.

Of the three distinct excitatory synaptic signals in B cells of the 9th and 10th paravertebral sympathetic ganglia of the frog, the fast nicotinic e.p.s.p. is the best understood (Nishi & Koketsu, 1960; Blackman, Ginsborg & Ray, 1963; Kuba & Nishi, 1979; MacDermott, Conner, Dionne & Parsons, 1980). The fast e.p.s.p. is generated by a decrease in the membrane resistance similar to that found at skeletal nerve–muscle synapses and at some fast excitatory synapses of vertebrates and invertebrate central neurones. In contrast, an increased input resistance during the muscarinic e.p.s.p. and the late slow e.p.s.p. was reported by Weight & Votava (1970) and Schulman & Weight (1976), who suggested that the e.p.s.p.s were caused by an inactivation of a resting K^+ conductance (see also Kobayashi & Libet, 1970). Recently, a voltage-dependent K^+ conductance, called the M current, has been identified in ganglion cells; it is reduced when either muscarine or LHRH is added to the bathing solution (Adams & Brown, 1980; Adams *et al.* 1982). However, in about half of the cells that we have studied, the size of the inward late slow and the muscarinic e.p.s.c.s increased with hyperpolarization up to and beyond the equilibrium potential for K^+, as judged by the reversal of the action potential after-hyperpolarization. Similar results have been reported by Katayama & Nishi (1982) and Jan & Jan (1982) for the late slow e.p.s.p. and by Kobayashi & Libet (1968, 1974), Kuba & Koketsu (1974, 1976), and McCort, Nash & Weight (1982) for the muscarinic e.p.s.p.

The M current is inactivated at membrane potentials more negative than −60 mV, and we have confirmed using voltage steps that the relaxation of the current is absent at hyperpolarized potentials, but large synaptic currents were generated despite the inactivation of the M current. The possibility exists that the currents measured in the soma are produced by the activation of receptors in the axon far from the soma. However, single cells dissociated from ganglia and lacking axons are capable of generating the same range of responses to muscarinic and peptidergic agonists as are found for nerve-evoked responses in isolated ganglia.

Although a decrease in the K^+ conductance probably represents a major component of the slow e.p.s.p.s near the resting potential, it can only be a part of the ionic mechanism. In addition to finding conductance decreases we have measured conductance increases in some cells at hyperpolarized potentials. Kuba & Koketsu (1976) and Nishi & Katayama (1981) reporting a similar heterogeneity among muscarinic

and peptidergic responses have suggested that two conductance mechanisms may contribute. The diversity of voltage dependencies that we have observed in the e.p.s.c.s could be explained if the relative proportion of two or more mechanisms varied among cells.

The slow muscarinic and slow peptidergic e.p.s.c.s reversed polarity at hyperpolarized potentials in some cells, consistent with a mechanism dominated by a conductance decrease to K^+. In other cells there was no reversal and a conductance increase occurred at hyerpolarized potentials. The biphasic peptidergic response of the cell in Fig. 4 appears to be a mixture of these two types, and R. Stickgold (unpublished data) has studied similar muscarinic responses. In a few well-studied cells the response started out non-reversing but later could be reversed. This evidence suggests that the response types are not fixed and that a component of the response, probably the conductance increase, is labile. Both Na^+ and Ca^{2+} may contribute to the conductance increases (Kuba & Koketsu, 1976; Katayama & Nishi, 1977; Nishi & Katayama, 1981; Katayama & Nishi, 1982), but more work is needed to separate and characterize the components of the slow e.p.s.p.s. In particular, none of the data so far rules out the participation of other mechanisms, such as metabolic pumps (Torre, 1982).

The absence of a reversal in the synaptic current in some cells is consistent with the observation by Brown, Muller & Murray (1971) that for two opposing conductance changes the reversal potential cannot lie between the equilibrium potentials of the two ionic species producing the response. In the present circumstance the conductance changes are highly voltage-dependent, and even at a fixed membrane potential the ratio of the opposing conductance changes may vary during the response, as apparently occurs in Fig. 4.

Comparison with other slow responses

Adams, Parnas & Levitan (1980) have studied a long-lasting synaptic inhibition in neurone R15 of *Aplysia californicus*. During the first few minutes of the response its polarity can be reversed near the equilibrium potential for K^+, but in the later part, from 3–10 min following stimulation, it was not reversible over the range -40 mV to -120 mV. The explanation given for the non-reversible component of the response was a combined conductance increase to K^+ and decreased inward current carried by Na^+ or Ca^{2+}, the inverse of the hypothesis proposed for the slow e.p.s.p.s in bull-frog sympathetic neurones.

Peptidergic responses have been studied in other sympathetic ganglia. Repetitive stimulation of the hypogastric nerve of the guinea-pig produces a slow depolarization in neurones of the inferior mesenteric ganglion that is mimicked by the application of substance P (Neild, 1978; Dun & Karczmar, 1979; Konishi, Tsunoo & Otsuka, 1979). Conductance decreases have been reported during both the nerve-evoked depolarization and the depolarization to substance P, however, a combined conductance decrease to K^+ and a conductance increase to Na^+ has recently been found during these responses in some neurones (Minota, Dun & Karczmar, 1981; Dun & Minota, 1981; Dun & Miang, 1982), raising the possibility that peptidergic e.p.s.p.s in the inferior mesenteric ganglion may also have several components and a complexity similar to that found in bull-frog sympathetic neurones.

Similarities between the slow muscarinic and the slow peptidergic responses

In almost every neurone where both the muscarinic and the peptidergic responses were studied together, either to nerve stimulation or to agonist application, both responses had the same voltage dependence and exhibited the same conductance changes. This would be a remarkable coincidence if entirely different mechanisms were involved. The close parallel, however, does not rule out the possibility that the muscarinic receptors and the peptidergic receptors activate separate mechanisms that produce additive synaptic currents.

We have found that the muscarinic e.p.s.c. was generally reduced but not completely abolished during the peptidergic e.p.s.c. Since the degree of interaction depended on many factors, such as the amount of stimulation, it was not possible to determine whether the generating mechanisms for the muscarinic and peptidergic responses overlapped completely. However, when the size of the peptidergic response was increased by applying LHRH, it was possible in a few cells to occlude the response to bethanechol totally; conversely, a saturating response to bethanechol entirely occluded the response to LHRH. The ability of both the non-cholinergic transmitter and LHRH to reduce the muscarinic response is yet another example of their similar physiological actions.

Muscarinic and peptidergic receptors on a ganglion cell are activated by different transmitters released by different nerves and can be independently blocked. A part of the interaction between the nerve-evoked responses could be due to a reduced release of ACh by a presynaptic action of the peptidergic transmitter; however, the size of the fast e.p.s.c. does not decrease during the peptidergic response, hence a substantial decrease in the release of ACh does not occur, and in any case presynaptic actions cannot account for the interaction between the responses induced by bethanechol and LHRH. Because the full activation of one response can completely occlude the other, the effects of activating the post-synaptic muscarinic and peptidergic receptors probably converge onto a final common path. For example, they could share a common second messenger, as proposed for other slow synaptic responses (Hartzell, 1981).

The convergence of signals from independent receptors on the surface of a cell has also been invoked in the parotid gland of the rat (Putney, 1977; Gallacher & Petersen, 1980). The activation of either muscarinic, α-adrenergic, or peptidergic receptors increases the membrane conductance of parotid cells, apparently at the same sites since the response of the cell to activation of one type of receptor blocked the ability of the other two types to produce the same response. Another example of convergence is found in cultured mouse spinal neurones where responses to γ-aminobutyric acid (GABA) and glycine may lead to a common conductance increase for Cl^- (Barker & McBurney, 1979).

Differences between the slow muscarinic and the slow peptidergic responses

In a few cultured neurones the voltage dependence of the two responses differed, particularly during their early parts. Some of this discrepancy may be accounted for by differences in time course of the components of the two responses. For example, the early reversal of the response to LHRH in Fig. 12 might reflect a longer latency for a conductance-increase mechanism. If a conductance increase occurred earlier

in the response to bethanechol owing to different intermediate processes, then a reversal of the simultaneous conductance decrease could be masked.

The muscarinic and peptidergic responses have significantly different time courses when stimulated by a brief train of impulses: the peptidergic response reaches its peak amplitude about 1 min after stimulation, by which time the muscarinic response has subsided; however, when agonists are applied, the time courses of the muscarinic and peptidergic responses are comparable (Figs. 10 and 12).

Little is known about the delivery of the peptidergic transmitter following nerve stimulation. LHRH-like immunoreactivity has been found in synaptic boutons which surrounded the smaller neurones of the ganglia (Jan, Jan & Brownfield, 1980; Jan & Jan, 1982). The B cells we have studied tend to be larger than the C cells. If the peptide is released mainly near the C cells, then part of the slow time course of the peptidergic response may be caused by diffusion barriers impeding both access and removal of the transmitter substance. Consistent with this possibility, an LHRH analogue, which acts as an antagonist, shortens the time course of the nerve-evoked and LHRH-induced responses when applied at their peaks (Jan & Jan, 1982).

The nicotinic receptors on sympathetic ganglion cells of the frog are strongly concentrated under synaptic boutons (Marshall, 1981). Very little is known about the distribution of either muscarinic or peptidergic receptors on the surfaces of ganglion cells. Some if not most of these receptors are probably located on cell bodies since slow responses in cultured cell bodies were indistinguishable from those in intact ganglion cells.

Physiological role of the slow synaptic potentials

The slow muscarinic e.p.s.p. is activated by the same transmitter, ACh, and is elicited by stimulating the same nerve which produces the fast nicotinic e.p.s.p. The muscarinic effects of ACh only become appreciable after several stimuli, but they can last for up to 1 min. During the muscarinic e.p.s.p. a neurone is more excitable and the initiation of an impulse by a fast nicotinic e.p.s.p. is more certain (Schulman & Weight, 1976). Thus, transmission through the ganglion during prolonged stimulation at high frequency should be automatically enhanced by the muscarinic signal. Furthermore, the muscarinic response does not desensitize with prolonged application of ACh, and thereby tends to counteract any reduction of the nicotinic response.

The slow peptidergic e.p.s.p. lasts about ten times longer than the muscarinic e.p.s.p. Stimulation of the peptidergic axons with a train of impulses appears to increase the excitability of ganglion cells further through the same mechanisms which produce the muscarinic response; thus, the peptidergic pathway is an alternate but longer-lasting means for enhancing ganglionic transmission of fast nicotinic e.p.s.p.s. However, during a large peptidergic response the muscarinic response is less effective because the ionic mechanisms are shared. One possible advantage of this arrangement is to limit the maximum excitability of a cell while enabling either pathway to produce maximal enhancement. We have as yet no information about the function of the slow responses *in vivo*.

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REFERENCES

ADAMS, P. R. & BROWN, D. A. (1980). Luteinizing hormone-releasing factor and muscarinic agonists act on the same voltage-sensitive K^+-current in bullfrog sympathetic neurones. *Br. J. Pharmac.* **68**, 353–355.

ADAMS, P. R., BROWN, D. A. & CONSTANTI, A. (1982). M-currents and other potassium currents in bullfrog sympathetic neurones. *J. Physiol.* **330**, 537–572.

ADAMS, W. B., PARNAS, I. & LEVITAN, I. B. (1980). Mechanism of long-lasting inhibition in *Aplysia* neuron R15. *J. Neurophysiol.* **44**, 1148–1160.

BARKER, J. L. & MCBURNEY, R. N. (1979). GABA and glycine may share the same conductance channel on cultured mammalian neurons. *Nature, Lond.* **277**, 234–236.

BENNETT, M. V. L., MODEL, P. G. & HIGHSTEIN, S. M. (1976). Stimulation induced depletion of vesicles, fatigue of transmission and recovery processes at a vertebrate synapse. *Cold Spring Harb. Symp. quant. Biol.* **40**, 25–35.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963). Synaptic transmission in the sympathetic ganglion of the frog. *J. Physiol.* **167**, 355–373.

BROWN, D. A. & ADAMS, P. R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neuron. *Nature, Lond.* **283**, 673–676.

BROWN, J. E., MULLER, K. J. & MURRAY, G. (1971). Reversal potential for an electrophysiological event generated by conductance changes: mathematical analysis. *Science, N.Y.* **174**, 318.

DODD, J. & HORN, J. (1983). Muscarinic inhibition of sympathetic C neurones in the bullfrog. *J. Physiol.* **334**, 271–291.

DUN, N. J. & JIANG, Z. G. (1982). Non-cholinergic excitatory transmission in inferior mesenteric ganglia of the guinea-pig: possible mediation by substance P. *J. Physiol.* **325**, 145–159.

DUN, N. J. & KARCZMAR, A. G. (1979). Actions of substance P on sympathetic neurones. *Neuropharmacology* **18**, 215–218.

DUN, N. J. & MINOTA, S. (1981). Effects of substance P on neurones of the inferior mesenteric ganglia of the guinea-pig. *J. Physiol.* **321**, 259–271.

ECCLES, J. C. (1964). *The Physiology of Synapses*, Springer-Verlag, Berlin.

ECCLES, R. M. (1952). Responses of isolated curarized sympathetic ganglia. *J. Physiol.* **117**, 196–217.

ECCLES, R. M. & LIBET, B. (1961). Origin and blockade of the synaptic responses of curarized sympathetic ganglia. *J. Physiol.* **157**, 484–503.

ECKER, A. & WIEDERSHEIM, R. (1896). *Anatomie des Frosches*, revised by E. Gaupp, Vieweg, Braunschweig, vol. 2, p. 212.

GALLACHER, D. V. & PETERSEN, O. H. (1980). Substance P increases membrane conductance in parotid acinar cells. *Nature, Lond.* **283**, 393–395.

GINSBORG, B. K. (1976). Physiology of the autonomic nervous system. In *Frog Neurobiology*, ed. LLINAS, R. & PRECHT, W., pp. 151–168. Berlin: Springer-Verlag.

HARTZELL, H. C. (1981). Mechanisms of slow postsynaptic potentials. *Nature, Lond.* **291**, 539–544.

HARTZELL, H. C., KUFFLER, S. W., STICKGOLD, R. & YOSHIKAMI, D. (1977). Synaptic excitation and inhibition resulting from direct action of acetylcholine on two types of chemoreceptors on individual amphibian parasympathetic neurones. *J. Physiol.* **271**, 817–846.

HORN, J. P. & DODD, J. (1981). Monosynaptic muscarinic activation of K^+ conductance underlies the slow inhibitory postsynaptic potential in sympathetic ganglia. *Nature, Lond.* **292**, 625–627.

JAN, L. Y. & JAN, Y. N. (1982). Peptidergic transmission in sympathetic ganglia of the frog. *J. Physiol.* **327**, 219–246.

JAN, L. Y., JAN, Y. N. & BROWNFIELD, M. S. (1980). Peptidergic transmitters in synaptic boutons of sympathetic ganglia. *Nature, Lond.* **288**, 380–382.

JAN, Y. N., JAN, L. Y. & KUFFLER, S. W. (1979). A peptide as a possible transmitter in sympathetic ganglia of the frog. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1501–1505.

JAN, Y. N., JAN, L. Y. & KUFFLER, S. W. (1980). Further evidence for peptidergic transmission in sympathetic ganglia. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5008–5012.

KATAYAMA, Y. & NISHI, S. (1977). The ionic mechanism of the late slow EPSP in amphibian sympathetic ganglion cells. *Proc. int. Union physiol. Sci.* **13**, 371.

KATAYAMA, Y. & NISHI, S. (1982). Voltage-clamp analysis of peptidergic slow depolarizations in bullfrog sympathetic ganglion cells. *J. Physiol.* **333**, 305–313.

KATZ, B. (1969). *The Release of Neural Transmitter Substances*, Springfield: Charles C. Thomas.

KEHOE, J. & MARTY, A. (1980). Certain slow synaptic responses: their properties and possible underlying mechanisms. *A. Rev. Biophys. & Bioeng.* **9**, 437–465.

KOBAYASHI, H. & LIBET, B. (1968). Generation of slow postsynaptic potential without increases in ionic conductance. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1304–1311.

KOBAYASHI, H. & LIBET, B. (1970). Actions of adrenaline and acetylcholine on sympathetic ganglion cells. *J. Physiol.* **208**, 353–372.

KOBAYASHI, H. & LIBET, B. (1974). Is inactivation of potassium conductance involved in slow postsynaptic excitation of sympathetic ganglion cells? Effects of nicotine. *Life Sci., Oxford* **14**, 1871–1883.

KONISHI, S., TSUNOO, A. & OTSUKA, M. (1979). Substance P and noncholinergic excitatory synaptic transmission in guinea-pig sympathetic ganglia. *Proc. imp. Acad. Japan.* **55**B, 525–530.

KRNJEVIĆ, K. (1974). Chemical nature of synaptic transmission in vertebrates. *Physiol. Rev.* **54**, 418–540.

KUBA, K. & KOKETSU, K. (1974). Ionic mechanism of the slow excitatory postsynaptic potential in bullfrog sympathetic ganglion cells. *Brain Res.* **81**, 338–342.

KUBA, K. & KOKETSU, K. (1976). Analysis of the slow excitatory postsynaptic potential in bullfrog sympathetic ganglion cells. *Jap. J. Physiol.* **26**, 647–664.

KUBA, K. & KOKETSU, K. (1978). Synaptic events in sympathetic ganglia. *Prog. Neurobiol.* **11**, 77–169.

KUBA, K. & NISHI, S. (1979). Characteristics of fast excitatory postsynaptic current in bullfrog sympathetic ganglion cells. *Pflügers Arch.* **378**, 205–212.

KUFFLER, S. W. (1980). Slow synaptic responses in autonomic ganglia and the pursuit of a peptidergic transmitter. *J. exp. Biol.* **89**, 257–286.

KUNO, M. (1971). Quantal aspects of central and ganglionic synaptic transmission in vertebrates. *Physiol. Rev.* **51**, 647–678.

LAPORTE, Y. & LORENTE DE NO, R. (1950). Potential changes evoked in a curarized sympathetic ganglion by presynaptic volleys of impulses. *J. cell. comp. Physiol.* **35**, 61–106.

LIBET, B. (1979). Slow synaptic actions in ganglionic functions. In *Integrative Functions of the Autonomic Nervous System*, ed. BROOKS, C. MCC., KOIZUMI, K. & SATO, A., pp. 197–222, Tokyo: University of Tokyo Press.

LIBET, B., CHICHIBU, S. & TOSAKA, T. (1968). Slow synaptic responses and excitability in sympathetic ganglia of the bullfrog. *J. Neurophysiol.* **31**, 383–395.

LLINAS, R. & NICHOLSON, C. (1976). Reversal properties of climbing fibre potential in cat Purkinje cells: An example of a distributed synapse. *J. Neurophysiol.* **39**, 311–323.

MCCORT, S. M., NASH, J. W. & WEIGHT, F. F. (1982). Voltage-clamp analysis of slow muscarinic excitation in sympathetic neurons of bullfrog. *Neurosci. Abstr.* **8**, 501.

MACDERMOTT, A. B., CONNER, E. A., DIONNE, V. E. & PARSONS, R. L. (1980). Voltage clamp study of fast excitatory synaptic currents in bullfrog sympathetic ganglion cells. *J. gen. Physiol.* **75**, 39–60.

MCMAHAN, V. J. & KUFFLER, S. W. (1971). Visual identification of synaptic boutons on living ganglion cells and of varicosities in postganglionic axons in the heart of the frog. *Proc. R. Soc.* B **177**, 485–508.

MARSHALL, L. M. (1981). Synaptic localization of α-bungarotoxin binding which blocks nicotinic transmission at frog sympathetic neurones. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1948–1952.

MINOTA, S., DUN, N. J. & KARCZMAR, A. G. (1981). Substance P depolarization of sympathetic neurons: not simple K^+ inactivation. *Brain Res.* **216**, 224–228.

NEILD, T. O. (1978). Slowly-developing depolarization of neurons in the guinea-pig inferior mesenteric ganglion following repetitive stimulation of the preganglionic nerves. *Brain Res.* **140**, 231–239.

NISHI, S. (1974). Ganglionic transmission. In *The Peripheral Nervous System*, ed. HUBBARD, J. I., pp. 225–255. New York: Plenum Press.

NISHI, S. & KATAYAMA, Y. (1981). The non-cholinergic excitatory transmission in sympathetic ganglia. In *Adv. Physiol. Soc. Vol. 4. Physiology of Excitable Membranes*, ed. SALANKI, J., pp. 323– 327. London: Pergamon Press.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *J. cell. comp. Physiol.* **55**, 15–30.

NISHI, S. & KOKETSU, K. (1968). Early and late afterdischarges of amphibian sympathetic ganglion cells. *J. Neurophysiol.* **31**, 109–118.

NISHI, S., SOEDA, H. & KOKETSU, K. (1965). Studies on sympathetic B and C neurons and patterns of preganglionic innervation. *J. cell. comp. Physiol.* **66**, 19–32.

PICK, J. (1957). Sympathectomy in amphibians. *J. comp. Neurol.* **107**, 169–207.

PUTNEY, J. W. (1977). Muscarinic, alpha-adrenergic and peptide receptors regulate the same calcium influx sites in the parotid gland. *J. Physiol.* **268**, 139–149.

RIVIER, J. E. & VALE, W. W. (1978). [D-pGlu[1], D-Phe[2], D-Trp[3,6]]-LRF. A potent luteinizing hormone releasing factor antagonist *in vitro* and inhibitor of ovulation in the rat. *Life Sci., Oxford* **23**, 869–876.

SCHULMAN, J. A. & WEIGHT, F. F. (1976). Synaptic transmission: long-lasting potentiation by a postsynaptic mechanism. *Science, N.Y.* **194**, 1437–1439.

SEJNOWSKI, T. J. (1982). Peptidergic synaptic transmission in sympathetic ganglia. *Fedn Proc.* **41**, 2923–2928.

SEJNOWSKI, T. J. & KUFFLER, S. W. (1981). Integration of peptidergic and muscarinic synaptic transmission in amphibian sympathetic ganglion neurons. *Neurosci. Abstr.* **7**, 726.

SKOK, V. I. (1973). *Physiology of Autonomic Ganglia.* Tokyo: Igaka Shoin Ltd.

TAKEUCHI, A. (1978). Junctional transmission. I. Postsynaptic mechanisms. In *Handbook of Physiology – The Nervous System I*, ed. BROOKHART, J., MOUNTCASTLE, V., HANDEL, E. & GEIGER, S., pp. 295–327. Bethesda, Maryland: American Physiological Society.

TORRE, V. (1982), The contribution of the electrogenic sodium–potassium pump to the electrical activity of toad rods. *J. Physiol.* **333**, 315–341.

VOLLE, R. L. (1975). Cellular pharmacology of autonomic ganglia. In *Cellular Pharmacology of Excitable Tissues*, ed. NARAHASHI, R. pp. 89–140. Springfield: Charles C. Thomas.

WATANABE, H. (1980). Ultrastructural study of the frog sympathetic ganglia. In *Histochemistry and Cell Biology of Autonomic Neurons, SIF Cells, and Paraneurons*, ed. ERANKO, O. SOINILA, S. & PAIVARINTO, H., pp. 153–157. New York: Raven Press.

WEIGHT, F. F. (1982). Synaptic mechanisms in amphibian sympathetic ganglia. In *Autonomic Ganglia*, ed. ELFVIN, L.-G. New York: John Wiley & Sons.

WEIGHT, F. F. & VOTAVA, J. (1970). Slow synaptic excitation in sympathetic ganglion cells: evidence for synaptic inactivation of potassium conductance. *Science, N.Y.* **170**, 755–758.

WEIGHT, F. F. & WEITSEN, H. A. (1977). Identification of small intensely fluorescent (SIF) cells as chromaffin cells in bullfrog sympathetic ganglia. *Brain Res.* **128**, 213–226.

WEITSEN, H. A. & WEIGHT, F. F. (1977). Synaptic innervation of sympathetic ganglion cells in the bullfrog. *Brain Res.* **128**, 197–211.

EXPLANATION OF PLATE

Live B neurone in a lumbar paravertebral sympathetic ganglion of the bull-frog. The nucleus and smaller nucleolus are clearly visible using Nomarski differential-interference-contrast optics. Two pipette tips positioned above the cell were used for delivering agonists by pressure application; the lower micropipette was used for intracellular recording and current injection. The flow of the bathing solution was from the bottom of the photograph toward the two pipettes at the top. The cell body is about 50 μm in diameter.