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THE VERSATILE SYNAPSE

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SUMMARY

'Typically' chemical synaptic transmission takes place when an influx of calcium ions during a presynaptic nerve impulse triggers exocytosis of neurotransmitter substance from synaptic vesicles. The neurotransmitter diffuses across the synaptic cleft and occupies receptors embedded in the subsynaptic membrane. This interaction (directly or *via* a second messenger) operates characteristic ion channels and produces an increase in the postsynaptic membrane permeability to particular ions. Depending on the ionic species to which the postsynaptic membrane becomes more permeable, the physiological response will be an excitatory or an inhibitory postsynaptic potential. The action of neurotransmitters may be terminated either by enzymic inactivation or by cellular uptake mechanisms.

Over the last decade it has become clear that a neurotransmitter substance may exert a number of different actions on a single postsynaptic neurone. These may involve opening or closure of either voltage-independent or voltage-dependent ion channels. It is also possible that in some instances transmitters may act on neuronal biochemical systems to modify the physiology of postsynaptic cells without directly altering their electrical characteristics.

Analysis of the postsynaptic actions of neurotransmitter substances has become further complicated by the increasing body of evidence which indicates that more than one transmitter substance (one of which may be a peptide) can be released from a single presynaptic neurone. The significance of such dual transmitter systems has yet to be fully elucidated.

The efficacy of transmission across many synapses may be modified by either presynaptic or postsynaptic mechanisms; both transmitter release and postsynaptic responsiveness may depend on the recent history of a single synapse, on synaptic inputs from other neurones or on circulating neuroactive substances.

INTRODUCTION

Communication between neurones mainly takes place *via* either electrical or chemical synapses. Electrical synaptic transmission is associated with 'gap' junctions possessing intercellular channels which allow small molecules and ions to pass between cells. Movement of ions through these channels allows electrical current to flow between neurones; at some electrical junctions current can flow equally well in either direction, while other junctions show rectification. Generally, electrical synaptic

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transmission shows only relatively limited modification under different physiological conditions. Chemical synaptic transmission is more complex than electrical transmission, since the presynaptic neurone must possess mechanisms for synthesis, storage and release of transmitter substance, while the postsynaptic neurone must have the ability to respond to released transmitter. Transmitter substances can produce a wide range of different actions on postsynaptic neurones. The efficacy of chemical synaptic transmission may be considerably altered by either presynaptic or postsynaptic changes. The term 'modulation' has become popular in the neuroscience literature to describe a number of different alterations in transmission which cannot be explained in terms of 'classical' synaptic physiology; agents which bring about these changes have been called 'neuromodulators' or simply 'modulators'. Events that have been described as modulation include long-lasting synaptic responses, alterations in the effectiveness of transmitters mediated by agents that alone produce only minor effects on synaptic transmission or changes mediated by agents released from distant sites. However, 'modulation' in many cases is achieved by mechanisms which are merely an extension of the basic principles governing transmission across 'conventional' synapses.

NEUROTRANSMITTERS

For many years most research concentrated upon the 'classical' neurotransmitters [for example, acetylcholine (ACh), monoamines and amino acids] which are all relatively low molecular weight compounds. However, over the last few years application of immunohistochemical techniques has demonstrated that a wide range of peptides are localized within neurones (Hokfelt *et al.* 1980). The list of peptides found within the mammalian brain includes a number of hormones which had previously been considered to be exclusively associated with the pituitary or the gastrointestinal tract (Table 1). Immunoreactivity to a number of these vertebrate peptides has also been demonstrated in central neurones of a number of species of invertebrates. However, care is required in the interpretation of data obtained

Table 1. *Some peptides present in the mammalian nervous system*

Hypothalamic peptides	Thyrotropin-releasing hormone (TRH)
	Luteinizing hormone-releasing hormone (LHRH)
	Somatostatin
	Vasopressin
Pituitary hormones	Adrenocorticotrophic hormone (ACTH)
	α-Melanocyte-stimulating hormone (α-MSH)
Gut-brain peptides	Insulin
	Glucagon
	Cholecystokinin
	Vasoactive intestinal polypeptide (VIP)
	Methionine enkephalin
	Leucine enkephalin
	Substance P
	Neurotensin
Other peptides	Angiotensin II
	β-Endorphin
	Bradykinin

by immunohistochemistry, since antibodies may react with only one portion of a peptide, allowing cross-reactions to occur between peptides sharing similar amino acid sequences. This problem makes it particularly difficult to interpret the results of experiments in which antibodies to peptides from one species are used to determine the distribution of immunoreactivity in the nervous system of an unrelated species (for example, when antibodies to mammalian peptides are applied to invertebrate nervous systems). Under these circumstances it is therefore important to demonstrate, by independent techniques, that the peptide of interest is actually present.

Although peptides may be involved in synaptic transmission it should be remembered that, unlike the 'classical' neurotransmitters, they must be synthesized in the cell body, then carried by axonal transport to nerve terminals. Once released at the synapse, it is unlikely that they can be taken back into the presynaptic terminal for recycling (Fig. 1). As a result, it is probable that the supply of peptide in nerve

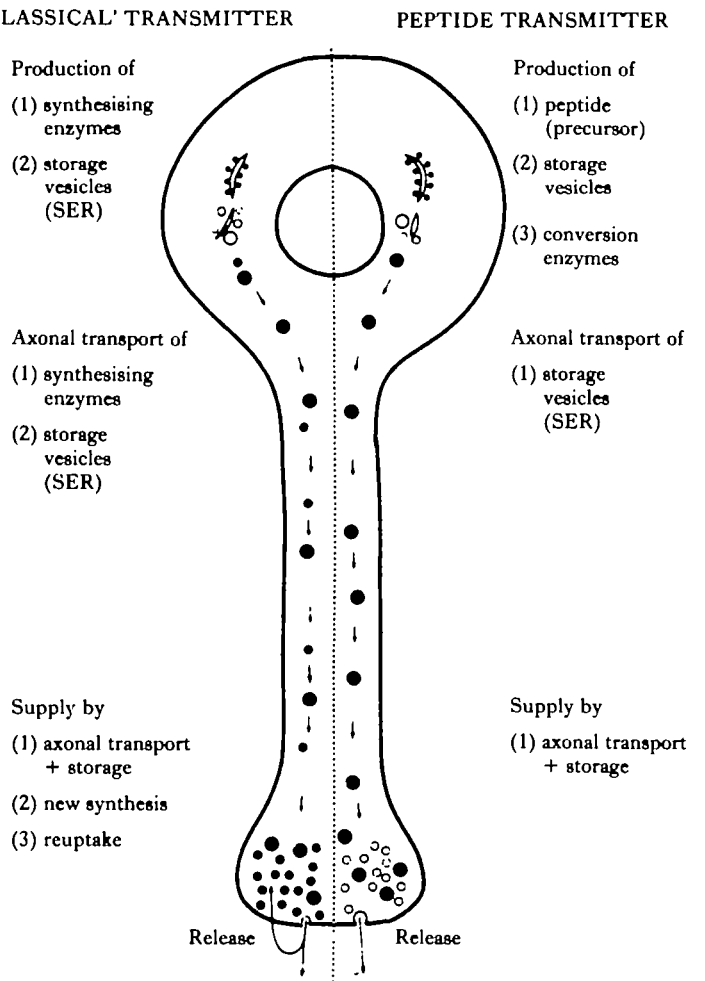

Fig. 1. Diagram showing some of the differences between the handling of a 'classical' transmitter and a peptide transmitter (SER, smooth endoplasmic reticulum). (From Hökfelt *et al.* 1980.)

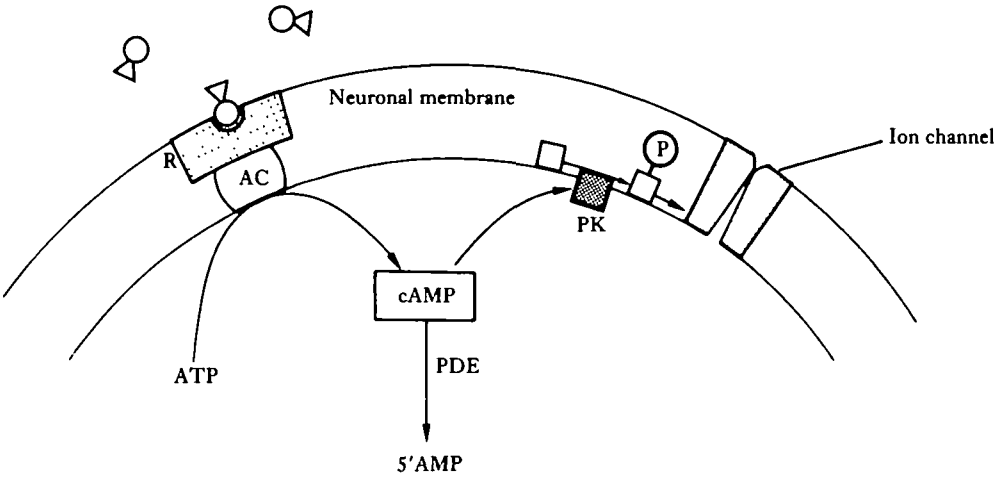

Fig. 2. Diagram showing the main steps by which cyclic AMP may operate as a second messenger in neurones. Transmitter molecules interact with receptors (R) on the neuronal membrane. This interaction stimulates adenylate cyclase (AC) to catalyse synthesis of cyclic AMP from ATP. Cyclic AMP (cAMP) activates a protein kinase (PK) which, in turn, may activate a membrane protein (P) involved in operating ion channels. Cyclic AMP is degraded by the enzyme phosphodiesterase (PDE).

terminals will be more prone to depletion during periods of elevated neuronal activity than the supply of a 'classical' neurotransmitter.

Interaction of transmitter substances with some receptors appears to have a direct effect upon ion channels, while other ion channels are operated indirectly by an intracellular 'second messenger'. One such messenger appears to be cyclic adenosine 3′,5′-monophosphate (cyclic AMP), which has been implicated in a number of post-synaptic responses (see Nathanson, 1977; Kupfermann, 1980). The precise mechanism by which cyclic AMP brings about its effects in neurones has not been elucidated. It seems, however, that transmitter-receptor interaction activates an adenylate cyclase which converts adenosine triphosphate (ATP) into cyclic AMP. This activates a protein kinase which, in turn, phosphorylates specific proteins. The phosphorylated protein may form part of an ion channel which is operated by the transmitter. Cyclic AMP is degraded by phosphodiesterases (Fig. 2). Besides operating ion channels, cyclic AMP appears to be involved in a number of other cellular functions including the regulation of transmitter synthesis and release. Cyclic guanine 3′,5′-monophosphate (cyclic GMP) may also act as a second messenger, however, the biochemical steps through which it operates are not entirely clear.

TRANSMITTER RELEASE

The efficacy of synaptic transmission may be controlled, at least in part, by changes in the amount of neurotransmitter substance released from presynaptic nerve terminals. The release of transmitter substances from neurones, like the exocytotic release of other secretory products, is apparently controlled by the cytoplasmic calcium concentration. Membrane potential probably exerts only a minor direct effect upon transmitter release at the vertebrate neuromuscular junction (Katz & Miledi, 1977)

and the squid giant synapse (Llinás, Steinberg & Walton, 1981), although presynaptic depolarization itself may have an important influence upon the amount of neurotransmitter released from crayfish motor neurone terminals (Dudel, Parnas & Parnas, 1983). The supply of transmitter available for release may also influence transmission.

Activity-dependent changes in release

The amount of neurotransmitter released by a single action potential will depend upon the preceding pattern of activity in the neurone. For example, junctional potentials in many crustacean muscle fibres show marked facilitation during trains of motor neurone action potentials (Dudel & Kuffler, 1961*a*) (Fig. 3), while repeated stimulation causes depression of postsynaptic responses at synapses made by sensory neurones on follower neurones both in the crayfish tail-flip escape neuronal circuit (Zucker, 1972) and in the gill-withdrawal reflex of *Aplysia* (Castellucci & Kandel, 1974). In each of the above examples, frequency-dependent alterations in synaptic transmission result from changes in the amount of transmitter released from presynaptic terminals rather than from a reduction in postsynaptic responsiveness (Zucker, 1973). The amount of transmitter released by each presynaptic impulse will depend both upon the number of synaptic vesicles immediately available for release and upon the efficiency of the release process. Both these parameters may be modified during repetitive activity to produce depression or enhancement of transmission. Many preparations show more than one type of frequency-dependent modification in synaptic transmission. At the frog neuromuscular junction there are three different components which contribute to enhancement of transmitter release caused by repetitive stimulation, each of which has a different decay time-constant. ('Facilitation' has a decay time-constant of 50–300 ms; 'augmentation' a time-constant of about 7 s, while 'potentiation' has a time-constant in tens of seconds or minutes.) Neuromuscular transmission is also modified by two other components: these have

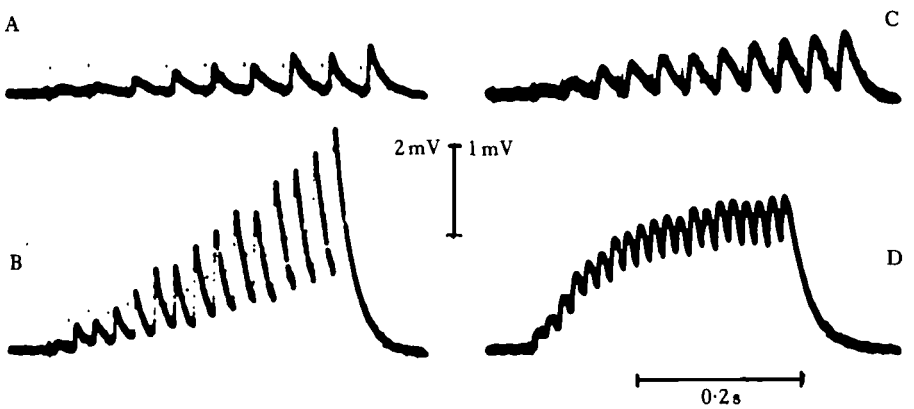

Fig. 3. Facilitation of junctional potentials at the crustacean neuromuscular junction. Traces A and B show facilitation of excitatory junctional potentials produced by stimulating an excitatory motor axon at 20 Hz and 40 Hz. Traces C and D show facilitation of inhibitory junctional potentials produced by stimulation at 23 Hz and 58 Hz. (The inhibitory junctional potentials produce depolarization because their equilibrium potential is slightly more positive than the muscle fibre resting potential.) (From Dudel & Kuffler, 1961*a*.)

been termed the 'expression factor', which selectively enhances 'augmentation' without influencing 'potentiation', and the 'time-constant factor', which specifically prolongs the decay of 'potentiation' (Magleby & Zengel, 1976). Presumably, different mechanisms underlie each of the above components. Depression, short-term facilitation, frequency facilitation and post-tetanic potentiation have all been observed at a cholinergic synapse on an identified *Aplysia* neurone (Schlapfer, Woodson, Tremblay & Barondes, 1974; Schlapfer, Tremblay, Woodson & Barondes, 1976). Each component has a characteristic time-course and may be produced by different patterns of presynaptic activity. These authors have attributed depression to initial depletion of readily-available transmitter. Frequency-facilitation (which develops during a stimulus train) and post-tetanic potentiation (observed after the end of a stimulus train) appear to result both from an increase in the supply of transmitter and an enhancement in the release process.

Although transmitter release may be influenced either by the efficiency of the release process or by the availability of transmitter, in most cases the presynaptic mechanisms underlying frequency-dependent modulation have not been completely elucidated. This is largely because it is not technically possible to make intracellular recordings from nerve terminals in most preparations. It has been suggested that frequency-dependent modulation of the process of transmitter release may be caused by a cumulative increase in intracellular free calcium which could be enhanced by a change in: (1) the amplitude of the presynaptic action potential, (2) the duration of the presynaptic action potential, (3) the point blockade of action potentials within presynaptic terminals.

Katz & Miledi (1965, 1968) obtained evidence that facilitation at the frog neuromuscular junction and the squid giant synapse results from a cumulative increase in the intracellular calcium concentration within the presynaptic nerve terminal. Although incomplete intracellular calcium buffering alone could account for facilitation observed at some junctions, the features of facilitation observed elsewhere indicate that calcium influx must be progressively enhanced in some way during a train of presynaptic action potentials (Bracho & Orkand, 1970; Lang & Atwood, 1973). In fact, non-linear calcium influx has been directly observed by injecting the calcium-sensitive protein aequorin into the cell bodies of *Aplysia* neurones (Stinnakre & Tauc, 1973; Fig. 4). In this preparation, enhanced calcium entry is associated with an increase in both action potential amplitude and duration.

Early evidence that the amplitude of the presynaptic spike determines the amount of transmitter released was largely based on the observation that presynaptic inhibition in sensory nerve fibres entering the spinal cord is associated with primary afferent depolarization (Eccles, 1964), while presynaptic facilitation is accompanied by primary afferent hyperpolarization (Mendell & Wall, 1964); transmitter release from motor neurone terminals is also enhanced by hyperpolarization (Hubbard & Willis, 1962). The amount of transmitter released at the squid giant synapse increases significantly with increase in the magnitude of depolarizing pulses applied to the presynaptic terminal (Katz & Miledi, 1967). In this preparation, sustained depolarization reduces the amplitude of presynaptic action potentials (by sodium inactivation) and decreases the size of postsynaptic potentials (Miledi & Slater, 1966; Weight & Erulkar, 1976); sustained hyperpolarization has the opposite effects.

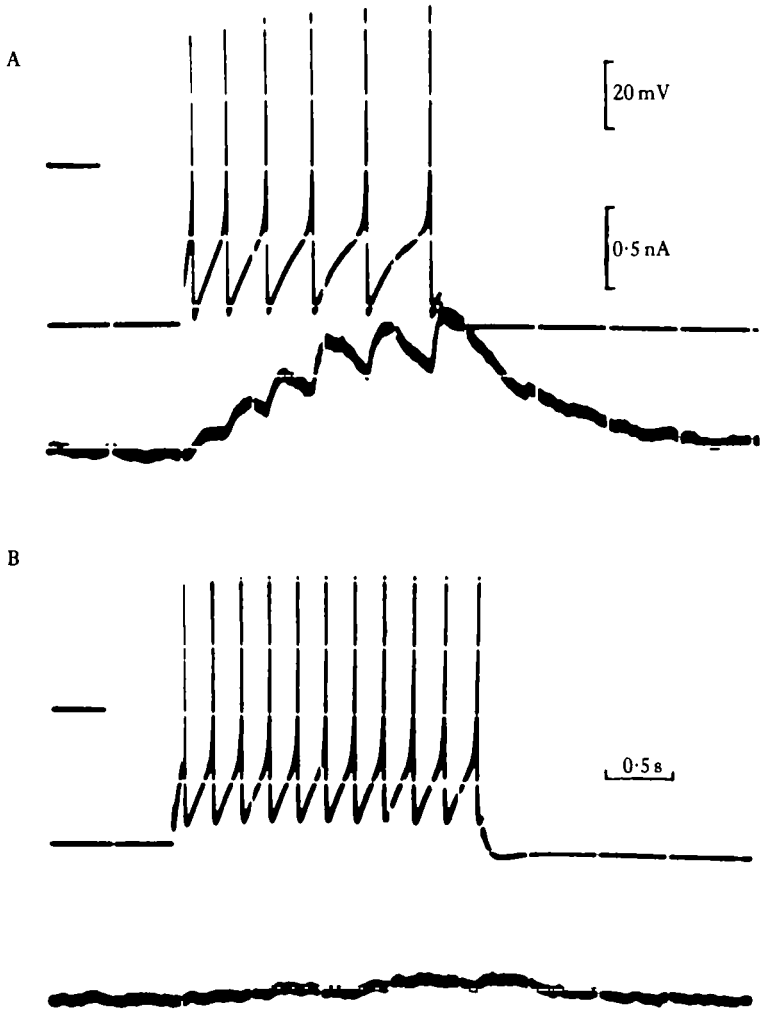

Fig. 4. Light emission from a molluscan neurone following injection of the calcium-sensitive protein aequorin. (A) There is a progressive increase in the size of the light response (lower trace) to each successive action potential in a burst. During a burst there is also a progressive increase in the spike overshoot and duration. (B) When calcium channels are blocked by adding Co^{2+} to the bathing medium, the light response is suppressed. (From Stinnakre & Tauc, 1973.)

However, a steady subthreshold presynaptic depolarization can enhance IPSPs recorded from an identified leech neurone in the absence of any significant change in action potential amplitude or duration (Nicholls & Wallace, 1978); hyperpolarization caused a reduction in IPSP amplitude. Similar results have been obtained with the crayfish neuromuscular junction; steady depolarization decreases the action potential amplitude, but produces a marked increase in the size of the postsynaptic potential (Wojtowicz & Atwood, 1983). At the beginning of a presynaptic depolarization, enhancement of evoked EPSPs developed more slowly than changes in the presynaptic membrane potential or action potential. It is, therefore, unlikely that either of these presynaptic changes are directly responsible for increased transmitter release in this preparation. Shapiro, Castellucci & Kandel (1980) observed similar modulatory

effects of membrane potential on transmitter release in *Aplysia* neurones, although in this case, polarization also caused changes in the action potential amplitude and duration. By voltage-clamping the presynaptic neurone they were able to demonstrate that transmitter release was modulated by a voltage-dependent steady state calcium current. Membrane depolarization also caused a reduction in outward potassium current; under physiological conditions this would also contribute to increased transmitter release by prolonging the action potential thereby increasing calcium influx. It is likely that a similar steady-state voltage-dependent calcium current may be responsible for graded transmission (in which transmitter release is altered by relatively small presynaptic membrane potential changes in the absence of action potentials) reported in both spiking (Nicholls & Wallace, 1978; Graubard, Raper & Hartline, 1980, 1983) and non-spiking neurones (Burrows, 1979; Burrows & Siegler, 1976, 1978; Bush & Cannone, 1973; Werblin & Dowling, 1969).

Broadening of successive presynaptic action potentials associated with a progressive increase in the intracellular Ca^{2+} concentration has been proposed as a possible mechanism underlying frequency-dependent changes in transmitter release in molluscs (Eckert & Lux, 1977; Stinnakre & Tauc, 1973). However, as discussed above, transmitter release from *Aplysia* neurones can be altered at different holding potentials without any change in the duration of transient presynaptic depolarizations (produced artificially by a voltage-clamp pulse). Zucker & Lara-Estrella (1979) have also shown that facilitation can occur at the crustacean neuromuscular junction under conditions which blocked changes in the duration of the action potential in motor neurone terminals. Nonetheless, although spike broadening may not be the only mechanism involved in frequency-dependent changes in transmitter release, any alteration in the duration of action potentials with a significant calcium component would almost certainly affect synaptic transmission.

Facilitation at the crustacean neuromuscular junction has been attributed to progressive invasion of motor neurone action potentials into motor neurone terminals (Lang & Atwood, 1973). Unfortunately, in this preparation, there is some doubt whether action potentials actually invade presynaptic terminals or whether they undergo pre-terminal block allowing only decremental electrotonic depolarization to reach transmitter release sites. Zucker (1974*a*,*b*), also studying the crustacean neuromuscular junction, obtained evidence that action potentials do reach motor neurone terminals. However, this question has been reopened recently, since Dudel (1983) (in patch-clamp recordings from the neuromuscular junctions of three other species of crustacean) reported that although some nerve terminals were electrically excitable, the majority were inexcitable.

Failure at axonal branch-points can selectively allow some terminal branches of a neurone to remain active while others no longer transmit. In some preparations, conduction failure may differentially affect individual synaptic targets, such as anatomically separate muscles (Grossman, Parnas & Spira, 1979*a*,*b*; Grossman, Spira & Parnas, 1973; Parnas, 1972), while in others, failure may affect some of the branches providing presynaptic input onto a single target (Hatt & Smith, 1976; Krnjević & Miledi, 1958*a*, 1959; Muller & Scott, 1981; Yau, 1976). Grossman *et al.* (1973, 1979*a*,*b*) and Parnas (1972) have demonstrated differential block at a branch-point of a crustacean motor neurone that innervates both the medial (DEAM) and

lateral (DEAL) bundles of the deep abdominal extensor muscles. At low stimulus frequencies (below 40–50 Hz), both the medial and lateral branches of the motor nerve conducted action potentials. At stimulus frequencies above 50 Hz, action potentials in the medial motor axon branch were rapidly blocked, while conduction in the lateral branch persisted. Conduction block takes place at branch points and largely results from an extracellular accumulation of potassium ions during action potential trains; this depolarizes the axon and reduces its input resistance and action potential amplitude. Grossman *et al.* (1979*b*) proposed that the two branches of the motor axon show differential conduction block because the concentration of sodium ions rises more rapidly in the smaller (lateral) branch owing to its larger surface-to-volume ratio. Elevation of the intracellular sodium concentration stimulates the sodium-pump, which will reduce extracellular potassium accumulation around the lateral motor axon branch and so prevent conduction block. If the sodium pump in these axons is electrogenic (Thomas, 1972), it may also enhance conduction through the axon branch by causing membrane potential hyperpolarization.

Repetitive stimulation causes synaptic depression by blocking conduction into terminals of mammalian (Krnjević & Miledi, 1958*a*, 1959) and crustacean (Hatt & Smith, 1976) motor neurones. Graded synaptic depression appears to result from a progressive increase in the number of terminals which become blocked as the presynaptic impulse frequency rises. Conduction failure occurs at intraganglionic branch-points of leech tactile sensory neurones (T cells) (Yau, 1976). Muller & Scott (1981) demonstrated that this failure causes a marked reduction in the size of post-synaptic responses in neurones to which the T cell is electrically coupled. Thus, branch-point block can allow frequency-dependent modification of transmission at electrical synapses, which normally exhibit only a limited degree of plasticity. In principle, synaptic inputs onto presynaptic areas of low safety factor, by altering membrane conductance, could exert a powerful influence on both chemical and electrical synaptic transmission (Spira, Yarom & Parnas, 1976).

Biochemical changes within the presynaptic terminal may alter the effectiveness of the actual release process as well as altering the supply of transmitter available for release. In some preparations, cyclic nucleotides appear to be involved in regulating transmitter synthesis and release, presumably producing their effects by stimulating phosphorylation of specific proteins (Castellucci *et al.* 1980; Kaczmarek *et al.* 1980). In addition to altering the magnitude of ion currents during the action potential (Klein & Kandel, 1978; Deterre, Paupardin-Tritsch, Bockaert & Gerschenfeld, 1981; see below), agents that increase intracellular cyclic AMP (such as cyclic AMP analogues, phosphodiesterase inhibitors) can cause an increase in the frequency of miniature end-plate potentials in vertebrate (Goldberg & Singer, 1969; Onodera, 1973; Statham & Duncan, 1976) and insect muscle (Fahim & Usherwood, 1983), suggesting that cyclic AMP can in some way influence spontaneous transmitter release. However, these data must be viewed with some caution, since theophylline and other methylxanthines cause release of intracellular calcium as well as inhibiting phosphodiesterases (Duncan & Statham, 1977).

Presynaptic receptors and transmitter release

Receptors for neurotransmitter substances have been demonstrated on the synaptic

terminals of many neurones (Starke, 1981). The list includes receptors for biogenic amines, acetylcholine, amino acids, prostaglandins, adenosine, ATP and a variety of peptides. Many types of neurone have receptors for their own neurotransmitter on their synaptic terminals (known as autoreceptors). Stimulation of presynaptic receptors can, in different preparations, either facilitate or depress transmitter release.

Presynaptic receptors may allow the function of presynaptic terminals to be modified in three ways: (1) by neuro-active agents present in the neuronal environment (for example, hormones entering the nervous system from the circulation and perhaps also substances, released by neurones, which are able to diffuse through the neuropile); (2) by synaptic contacts from other neurones onto presynaptic terminals; or (3) by transmitter released from the terminals themselves.

Circulating adrenalin may enhance skeletal neuromuscular transmission in mammals by acting upon presynaptic adrenoreceptors, since both adrenalin and noradrenalin increase the magnitude of end-plate potentials without altering the magnitude of muscle fibre responses to iontophoretically applied acetylcholine (Krnjević & Miledi, 1958*b*). It is also likely that circulating 5-hydroxytryptamine (5-HT) and octopamine presynaptically modulate neuromuscular transmission in crustaceans, where they act as neurohormones (Kravitz *et al.* 1980). 5-HT, in addition to acting postsynaptically on muscle fibres, enhances transmitter release from both excitatory and inhibitory motor neurone terminals (Dudel, 1965; Glusman & Kravitz, 1982), while octopamine exerts a smaller presynaptic action which is apparently limited to excitatory motor neurones (Fischer & Florey, 1983; Kravitz *et al.* 1980). Johnston, Kravitz, Meiri & Rahamimoff (1983) have recently shown that adrenocorticotrophic hormone (ACTH) produces (albeit in relatively high concentrations) potentiation of acetylcholine release from frog motor neurone terminals which may last as long as 4 h.

The first clear demonstration that presynaptic receptors could alter transmitter release was at the crustacean neuromuscular junction, where it was shown that stimulation of an inhibitory motor neurone, or application of γ-amino-butyric acid (GABA), reduced the number of transmitter quanta released from excitatory motor neurone terminals (Dudel & Kuffler, 1961*b*). Since then, presynaptic inhibition has been observed in a number of other preparations; enkephalin, for example, is thought to exert at least some of its effects in the spinal cord by inhibiting the release of substance P from nociceptive sensory nerve terminals (Jessell & Iversen, 1977). Mudge, Leeman & Fischbach (1979) have shown that enkephalin and [D-Ala^2]enkephalin (DAEA) depress the release of substance P from chick dorsal root ganglion cells in culture. Although these peptides have no detectable effect upon resting potential or membrane resistance, they cause a reduction in the duration of the action potential in the cell bodies of these neurones. This action of enkephalin and DAEA probably results from suppression of a calcium current. Enkephalin (or an enkephalin-like peptide) released from preganglionic nerve fibres also acts presynaptically to suppress cholinergic transmission through sympathetic ganglia (Konishi, Tsunoo & Otsuka, 1981). Presynaptic receptors on the terminals of some neurones can produce relatively long-term heterosynaptic facilitation of transmitter release; this has been studied extensively as the cellular basis of behavioural sensitization (Kandel, Brunelli, Byrne & Castellucci, 1976; see below).

Autoreceptors have been described which may either cause facilitation or

depression of transmitter release. Although it has been suggested that these receptors may, in some way, regulate neurotransmitter synthesis and release, there is still considerable doubt about their physiological role.

POSTSYNAPTIC ACTIONS

At 'conventional' synapses interaction of neurotransmitter with its postsynaptic receptors causes a rise in the conductance of the postsynaptic membrane by increasing the probability that particular ion channels will be open. The receptor-operated ion channels may be selective for sodium, potassium, calcium or chloride ions or may allow passage of more than one ion species. At some synapses, a single transmitter can interact with more than one type of receptor and produce several different conductance changes each with its own characteristic time-course (e.g. ACh in sympathetic ganglia, Kuba & Koketsu, 1978). A single presynaptic neurone can also generate a wide range of responses on different follower cells (e.g. in *Aplysia* neurones, Kandel & Wachtel, 1968).

Although receptor-operated ion channels act as current generators in neuronal membranes, the voltage shift produced by an EPSP is more important than the actual current which flows, since excitation requires that sufficient depolarization occurs to reach the threshold potential for action potential initiation. The amplitude of an EPSP is approximated by the following equation:

$$\Delta v_{EPSP} = \Delta g(E_{EPSP} - V_m)R_m .$$

Where Δv_{EPSP} is the change in membrane potential produced by the EPSP, Δg is the change in conductance produced by the EPSP, E_{EPSP} is the equilibrium potential of the EPSP, V_m is the membrane potential, $(E_{EPSP}-V_m)$ corresponds to the driving force on ions producing the EPSP and R_m is the membrane resistance of the neurone (Kuno, 1971). Therefore, if the membrane resistance of a neurone falls, the amplitude of EPSPs recorded in the neurone will also fall. Conversely, an increase in membrane resistance will be accompanied by an increase in EPSP amplitude. IPSPs can exert their effect on neurones more by virtue of their influence upon membrane resistance than upon their effect on membrane potential; in some neurones, in which the chloride equilibrium potential is less negative than the resting potential, the IPSP may cause a small depolarization. However, since the IPSP is accompanied by a marked reduction in neuronal membrane resistance, it will cause a reduction in the amplitude of EPSPs and so reduce the likelihood that they will produce sufficient depolarization to reach spike threshold. Conversely, some synaptic responses involve an increase in membrane resistance (by causing channel closure). The effect of this is to increase the amplitude of conventional postsynaptic potentials generated through other inputs; summed EPSPs will reach threshold more readily.

Voltage-dependent ion channels

Although the effectiveness of receptors may be slightly affected by the membrane potential, opening and closure of many ion channels is virtually independent of voltage; transmitter will produce a similar conductance change over a range of membrane potentials (even though the direction of current flow may be changed). There are,

however, a number of receptor-operated ion channels that exhibit voltage sensitivity. Some of these channels contribute to ionic currents underlying the action potential, which, therefore, may be modified by transmitter action.

ACh and glutamate produce excitation when applied to mammalian cortical neurones. The ACh response, which is mediated by muscarinic receptors, is relatively slow by comparison with the action of glutamate. ACh increases the input resistance of cortical neurones, and causes a hump to appear on the falling phase of the action potential (Krnjević, Pumain & Renauld, 1971). The reversal potential of the ACh response is more negative than the resting potential and, unlike IPSPs, is unaffected by leakage of chloride ions from the recording microelectrode. It has been concluded, therefore, that ACh causes a reduction in potassium conductance. This action may be direct, or may result indirectly from suppression of a calcium current since these neurones may have a calcium-dependent potassium conductance (see Meech, 1976).

ACh also causes an increase in input resistance of frog sympathetic ganglion cells by acting upon muscarinic receptors (Weight & Votava, 1970); as in mammalian cortical neurones, ACh slows repolarization of the action potential probably by suppressing potassium conductance (Kuba & Koketsu, 1975, 1976). ACh may suppress sodium and calcium currents in these neurones since it causes a decrease in the rate of rise of sodium and calcium action potentials. Adrenalin and noradrenalin have similar effects on the action potential to those of ACh (Minota & Koketsu, 1977). Although these workers observed no change in the characteristics of action potentials recorded from the cell bodies of sensory neurones following application of ACh or adrenalin (Kuba & Koketsu, 1976; Minota & Koketsu, 1977), Dunlap & Fischbach (1978) reported that γ-aminobutyric acid (GABA), noradrenalin and 5-HT could all shorten the plateau on the falling phase of action potentials recorded from cultured dorsal root ganglion cells. This could have resulted from suppression of a calcium current or activation of a potassium current. Ba^{2+}, which blocks potassium channels, did not prevent action potential shortening; it was concluded, therefore, that these drugs act directly to close calcium channels.

The increase in input resistance of sympathetic ganglion cells produced by muscarinic agonists is accompanied by an enhancement of repetitive firing attributable to suppression of a potassium current (Kuba & Koketsu, 1976; Weight & Votava, 1970). The characteristics of the muscarine-sensitive current (M-current) have been studied in more detail using voltage-clamp (Brown & Adams, 1980; Brown, Constanti & Adams, 1981). The M-current is activated at membrane potentials between about -60 mV and -10 mV and makes a steady contribution to the resting potential. Since the reversal potential of this current is normally between -80 mV and -100 mV, and is shifted by about 57 mV for a ten-fold change in external potassium concentration, it appears to be a potassium current. It is not a calcium-activated potassium current, since it is unaffected by Co^{2+}, Ni^{2+}, Mn^{2+} or Cd^{2+} which block calcium currents. Since the M-current constitutes an outward current that is turned on as the membrane potential becomes less negative, it would normally tend to oppose depolarizations of synaptic or other origin. Muscarinic agonists applied to the preparation, or synaptically released ACh, turn off the M-current. Consequently, in addition to causing some depolarization, they enhance neuronal excitability through a reduction in the outward current flowing through the membrane. The M-current is not only sensitive to

holinergic agents, but can also be operated by luteinizing hormone releasing hormone (LHRH); there is evidence that an LHRH-like peptide is released from some preganglionic nerve fibres and produces a long-lasting EPSP in ganglion cells (Jan, Jan & Kuffler, 1979, 1980). Responses of sympathetic neurones, however, may be complex since there is considerable heterogeneity among actions of both muscarinic agents and LHRH observed in different neurones; other currents may be involved in addition to the M-current (Kuffler & Sejnowski, 1983). Despite variations in the characteristics of responses of different neurones, muscarinic and peptidergic responses recorded from the same neurone have a similar voltage-dependence and produce similar conductance changes (even though the time-course of muscarinic and peptidergic EPSPs differ considerably). Although pharmacological evidence indicates that muscarinic agents act on different receptors from LHRH and its analogues, responses to LHRH could be blocked by application of muscarinic agonists and *vice versa*. This suggests that ACh and the LHRH-like peptide may operate the same ion channels *via* different receptors in sympathetic neurones.

The actions of 5-HT have been studied on several different molluscan preparations, where it is able to exert a range of voltage-dependent actions. Pellmar & Wilson (1977) used voltage-clamp to show that 5-HT exerted an excitatory effect with two components when applied iontophoretically to the cell bodies of neurones in the abdominal and buccal ganglia of *Aplysia*. The first of these actions was a conventional voltage-independent increase in sodium conductance, which was blocked in sodium-free external solutions. The second component was also depressed by sodium-free bathing solutions, but only developed at membrane potentials more positive than approximately −30 mV; the magnitude of the response increased with further

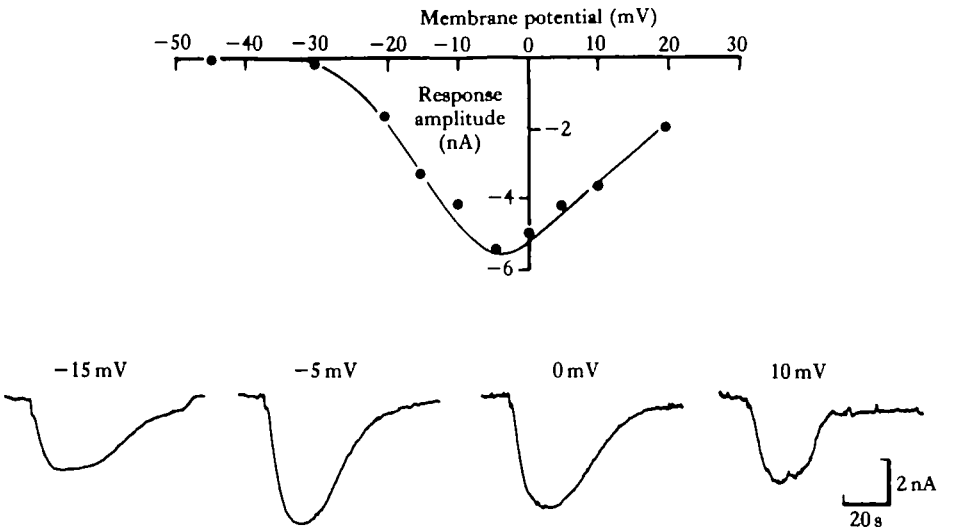

Fig. 5. Graph showing the voltage-sensitivity of the response to 5-HT recorded from an identified *Aplysia* neurone under voltage-clamp. At membrane potentials more negative than −30 mV, the response is absent. On depolarization, the magnitude of the response increases, reaching a peak at a membrane potential of about −5 mV. Lower traces are sample current responses to 5-HT at different membrane potentials. (From Pellmar, 1981.)

depolarization. Since the voltage-dependent component of the 5-HT response was unaffected by changes in the external potassium or chloride concentration, it was initially concluded that 5-HT probably activated a voltage-dependent increase in inward sodium current (Pellmar & Wilson, 1977). Further studies on the same preparation (Pellmar & Carpenter, 1979, 1980), however, showed that cobalt, manganese and cadmium ions rapidly and reversibly blocked the voltage-dependent component of the 5-HT response. Since these ions block calcium currents in other systems, these workers concluded that 5-HT induces a regenerative calcium current which is too slow to contribute to the action potential. The voltage sensitivity of this current is shown in Fig. 5.

Similar ionic currents can be generated synaptically in neurones of the land snails *Helix pomatia* and *Helix aspersa* (Cottrell, 1981, 1982*a,b*). Activation of an identified 5-HT-containing neurone (the giant serotonin neurone, GSN) produces a voltage-dependent synaptic response in an identified follower neurone (the A neurone) which is mimicked by 5-HT. The response consists of a prolonged small depolarization,

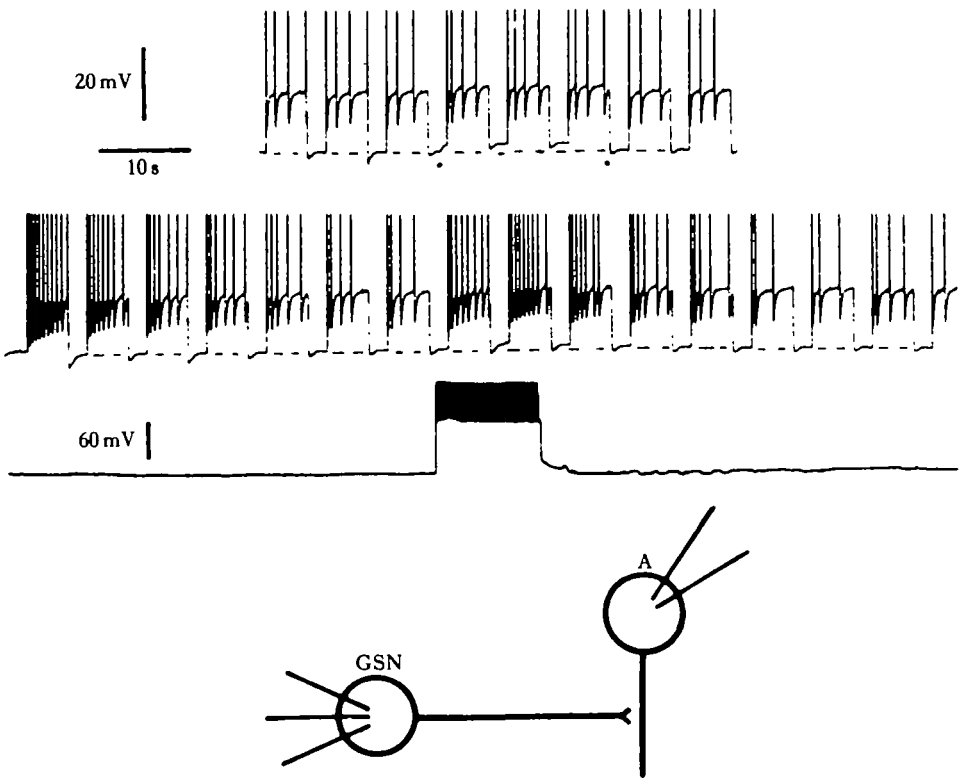

Fig. 6. Effect of activity in a molluscan serotonergic neurone (GSN) upon accommodation in a postsynaptic neurone (A). Middle trace: depolarizing current pulses applied to neurone A produce bursts of spikes. The number of action potentials evoked declines during successive current pulses. Activity in the GSN (bottom trace) causes a slight depolarization of the membrane potential and a marked increase in the number of action potentials evoked by each current pulse. Upper trace: this increase in excitability is not a direct result of membrane depolarization alone, since current injection (during the period marked by dots) does not mimic the effects of nerve stimulation upon excitability. (From Cottrell, 1982*b*.)

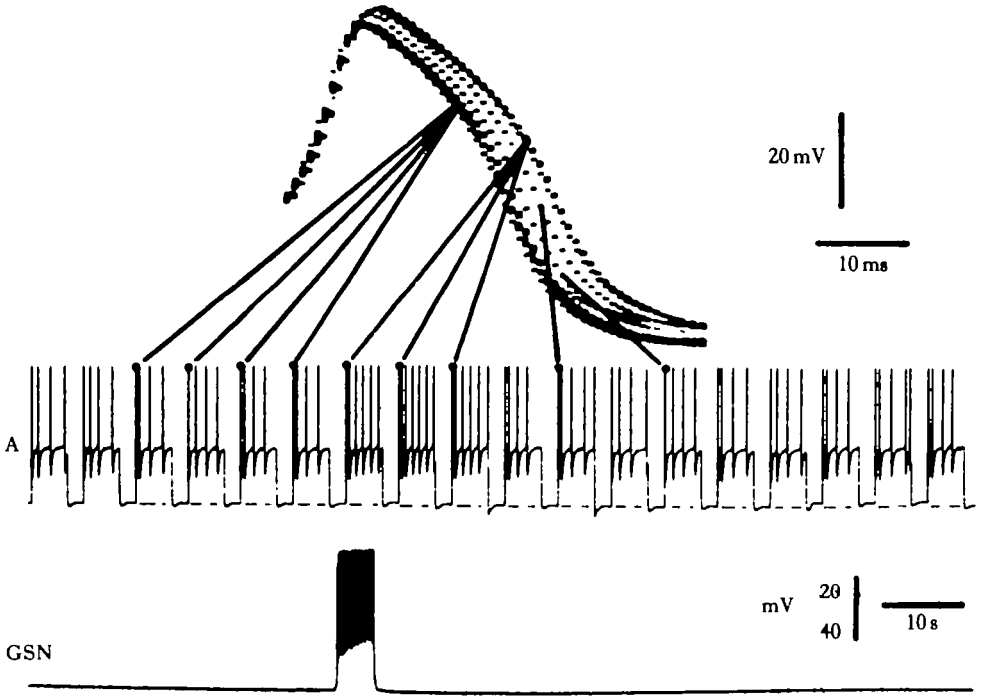

Fig. 7. Effect of activity in a serotonergic molluscan neurone (GSN) upon the duration of the action potential in a follower cell (A). The first A neurone action potential produced by each depolarizing current pulse is shown at a rapid sweep-speed (upper trace); activity in the GSN (lower trace) prolongs the falling phase of the A neurone spike. (From Cottrell, 1982*b*.)

which may be associated with small membrane potential oscillations and some spike activity in the postsynaptic neurone. Voltage-clamp analysis showed that although the response is small at normal resting potential values (about −40 to −50 mV), the response increases markedly at more positive holding potentials. Like the responses produced by 5-HT in *Aplysia* neurones (Pellmar & Wilson, 1977; Pellmar & Carpenter, 1979, 1980), the currents generated synaptically or by 5-HT in snail neurones are unaltered by removal of sodium or chloride ions from the bathing solution, but are depressed in the presence of cobalt or cadmium. The observations indicate that calcium is probably involved, although it is unclear whether it acts as a charge carrier itself or whether it controls the conductance of some other ion such as potassium. Although the functional significance of this voltage-dependent synaptically-mediated response is not entirely clear, activity in the giant serotonin neurone produces two changes which may be seen in voltage recordings from the postsynaptic A neurone. Firstly, it reverses accommodation of action potentials which occurs during repeated depolarizing current pulses (Fig. 6); this cannot be attributed simply to the depolarizing effect of synaptic current as it is not simulated by an equivalent increase in the amplitude of the applied current pulses. Secondly, it causes a prolongation of the falling phase of the action potential (Cottrell, 1982*a,b*; Fig. 7).

The gill withdrawal reflex in *Aplysia* has been used extensively to study the cellular basis of behavioural sensitization (Kandel *et al.* 1976); the response to tactile stimulation of the siphon undergoes prolonged sensitization following a strong stimulus

applied to the head. Sensitization results from presynaptic facilitation of transmitter release from the terminals of sensory neurones innervating the siphon. 5-HT mimics the effect of stimulating the sensitizing nerve pathway and appears to be the transmitter responsible for sensitization (Brunelli, Castellucci & Kandel, 1976). The effects both of stimulating the sensitizing pathway and of 5-HT are apparently mediated by an elevation in the intracellular concentration of cyclic AMP which, in turn, operates ion channels, by triggering phosphorylation of specific proteins (perhaps the ion channels themselves).

Because nerve terminals are inaccessible to electrophysiological techniques, sensory neurone somata have been studied as they appear to serve as a good model for sensory neurone terminals. Changes observed in calcium currents of the cell body action potential are sufficient to account for the changes associated with behavioural sensitization of the gill-withdrawal reflex in *Aplysia*. Both sensitization and 5-HT cause broadening of the action potential in the cell bodies of sensory neurones in the abdominal ganglion (Klein & Kandel, 1978). Initially this action potential broadening was thought to result from activation of voltage-dependent calcium channels. However, on closer examination, it turned out that 5-HT had no direct action on calcium channels at all, but instead caused a reduction in potassium conductance. The effect of this is to delay repolarization and so prolong the calcium current of the sensory neurone cell body (Klein & Kandel, 1980). More recently, the properties of 5-HT-sensitive potassium channels have been studied under patch-clamp (Siegelbaum, Camardo & Kandel, 1982). These channels are distinct from the four types of potassium channel that have been described in molluscan neurones (Adams, Smith

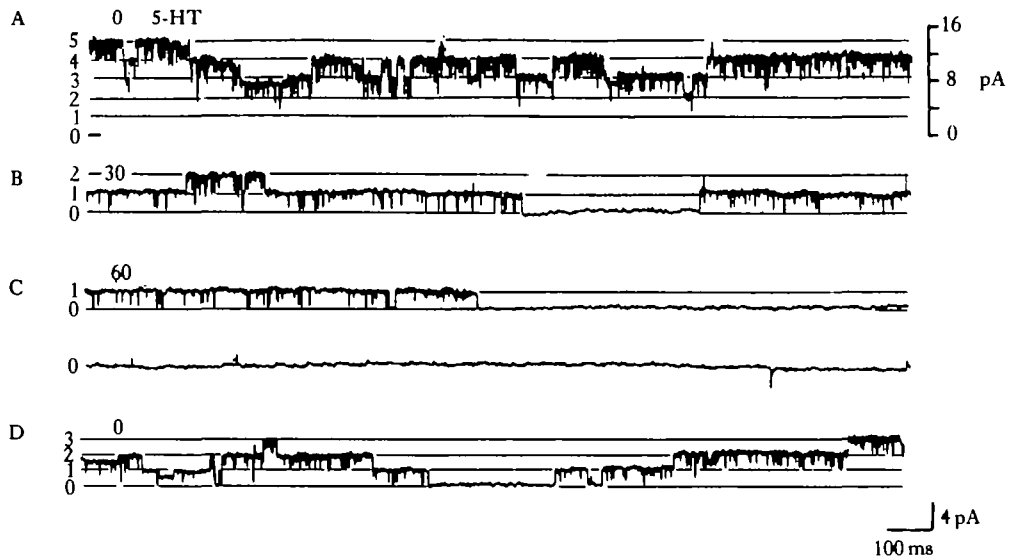

Fig. 8. Effect of 5-HT on single-channel currents recorded from sensory neurones of *Aplysia* using patch-clamp. (A) Currents recorded in the absence of 5-HT. (B) Recording after application of 30 μmol l^{-1} 5-HT to the bath. (C) Currents recorded in the presence of 60 μmol l^{-1} 5-HT. Upper and lower records are continuous. (D) Recording made after washing 5-HT from the preparation. Left-hand ordinate indicates the number of open channels. Right-hand ordinate shows the current. (From Siegelbaum, Camardo & Kandel, 1982.)

Thompson, 1980) and are the most frequently seen channel type in *Aplysia* sensory neurones; they are only slightly voltage-dependent and are insensitive to changes in the intracellular Ca^{2+} concentration (determined by using inside-out membrane patches). When 5-HT was applied in the external solution it caused a dose-dependent increase in the input resistance of the cell and a reduction in the number of active channels under the patch, without altering the kinetics of those potassium channels which continued to operate (Fig. 8). The seal at the tip of a patch electrode appears to be sufficiently tight to exclude pharmacological agents applied to the bathing solution from the membrane under the patch. 5-HT was effective when added to the bath during *in situ* patch experiments, indicating that it must exert its effect on ion channels indirectly *via* a second messenger, presumably cyclic AMP. This conclusion is supported by the fact that intracellular injection of cyclic AMP mimics the effects of 5-HT on single channels.

The molluscan peptide FMRFamide (Phe-Met-Arg-Phe-NH_2), like 5-HT, has voltage-dependent actions upon snail neurones (Cottrell, 1982*c*). At the resting potential, the peptide produced a hyperpolarizing response, which appears to result from an increase in potassium conductance. At potentials more positive than the resting potential, a second component to the peptide response became apparent; this consisted of an apparent inward current, which progressively increased in size with further depolarization. This was still present when sodium chloride in the external solution was replaced with sucrose, but was abolished when either Co^{2+} or Ba^{2+} were present. Injection of Ca^{2+}, on the other hand potentiated the peptide response. These observations suggest that the inward current induced by FMRFamide at relatively depolarized membrane potentials results from suppression of a calcium-dependent potassium current. The outward current observed at more negative membrane potentials probably results from opening of a different class of potassium channels. Recent patch-clamp recordings made at depolarized membrane potentials have revealed outward current channels. These single channel currents had the properties of potassium channels and appeared less frequently on application of FMRFamide to the surface of the cell body (Cottrell & Green, 1984). The physiological significance of two opposing voltage-dependent conductance changes evoked by a single transmitter is, as yet, unclear, although they would probably render the membrane potential less stable.

Some neuroactive substances exert specific effects upon molluscan burst firing neurones. Barker & Gainer (1974) and Barker, Ifshin & Gainer (1975) have studied the actions of the mammalian peptide hormones on identified neurosecretory neurones of *Aplysia* and the land snail *Otala*. Lysine vasopressin, arginine vasopressin and oxytocin induce burst firing in quiescent neurones or enhance burst formation in active cells; the axon hillock is more sensitive than the cell body to iontophoretically applied vasopressin. The change in neuronal firing pattern is long-lasting and persists after removal of the peptide. Voltage-clamp analysis showed that vasopressin causes the current-voltage (I/V) relationship of the neurone to develop a region of negative-slope resistance (Barker & Smith, 1976; Fig. 9). Since the magnitude of the response was dependent upon the external sodium concentration, these authors concluded that vasopressin probably induces a slowly inactivating, voltage-dependent sodium conductance, although they considered it possible that the net inward current could result

from alterations in potassium conductance. Although vasopressin and oxytocin are probably not found in molluscs, a peptide component which can induce burst activity has been isolated from the snail nervous system (Ifshin, Gainer & Barker, 1975).

ACh and dopamine can have the opposite effect to vasopressin on some molluscan bursting neurones; both inhibit a persistent slow regenerative inward current and suppress burst firing. Neurones L_2 to L_6 in the abdominal ganglion of *Aplysia* are monosynaptically inhibited by the cholinergic neurone L_{10}. This inhibition has a rapid phase which is reversed on hyperpolarization and results from an increase in chloride conductance. This is followed by a slow phase, lasting several seconds, which is not inverted by hyperpolarization. Because the slow phase failed to reverse, it had been attributed previously to synaptic activation of an electrogenic sodium pump, (Pinsker & Kandel, 1969) or to an increase in potassium conductance generated at some distance from the recording site (Kehoe & Ascher, 1970). Voltage-clamp has demonstrated that the slow phase of synaptic inhibition has no effect on the I/V curve of postsynaptic neurones at membrane potentials more negative than −60 mV. However, at more positive membrane potentials it eliminated the region of negative-slope resistance which is characteristic of burster neurones (Wilson & Wachtel, 1978; Fig. 10). The effects of synaptically-mediated inhibition could be mimicked by

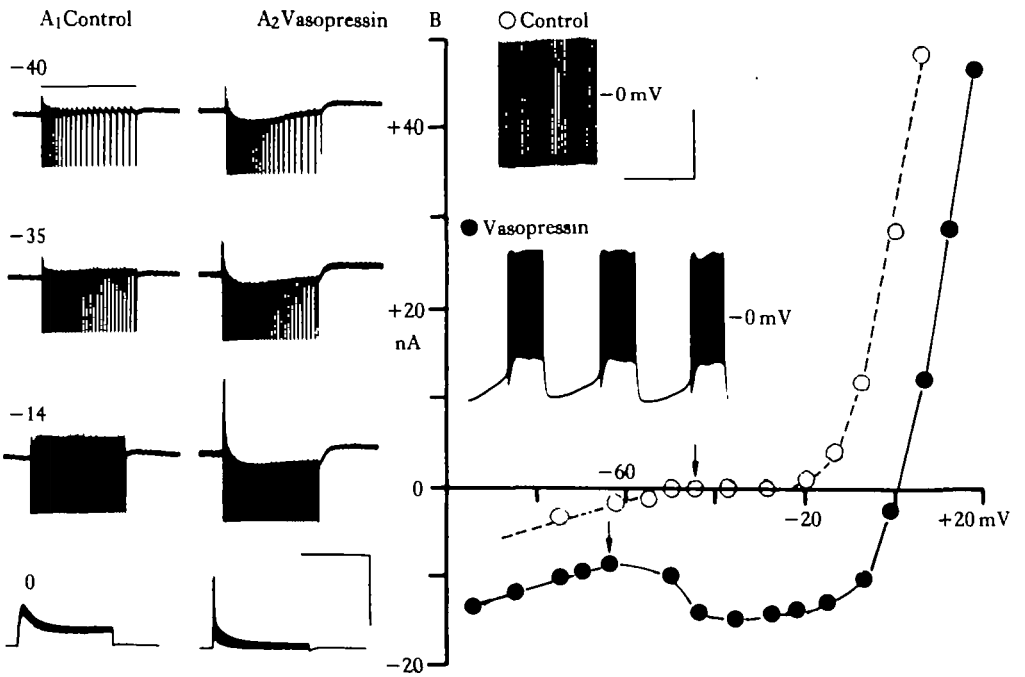

Fig. 9. Effect of vasopressin on the steady-state I/V relation in an identified molluscan neurone under voltage-clamp. (A_1) and (A_2) Currents produced by 5-s voltage command steps to the values indicated, for the duration of the horizontal bar at the upper left; rapid current deflections correspond to action potentials in unclamped regions of the axon; A_1, before and A_2, during exposure to vasopressin. (B) Graph obtained from currents in (A). Vasopressin (filled circles) causes a region of negative resistance to develop in the I/V curve. Inset in B shows membrane potential recordings from the neurone before and during application of vasopressin. Calibrations: (A) 10 nA (upper three traces) and 40 nA (bottom traces), 5 s; (B) 50 mV, 20 s. (From Barker & Smith, 1976.)

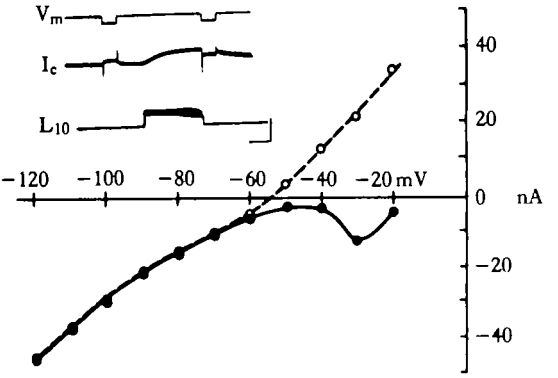

Fig. 10. Effect of prolonged cholinergic inhibition on the I/V curve of a molluscan burst firing neurone studied under voltage-clamp. Inset, the membrane potential of the burster neurone is held at −35 mV while 10 mV hyperpolarizing command pulses are applied before and just following activity in a cholinergic presynaptic neurone (L_{10}). The first command pulse produces an outward current (showing the existence of a negative resistance characteristic). The command pulse delivered during prolonged synaptic inhibition results in an inward current (indicating that the negative resistance has been suppressed). The graph illustrates the I/V relation of the neurone before (filled circles) and during (open circles) synaptic inhibition; the negative resistance region in the I/V curve is suppressed by cholinergic inhibition. Calibration: horizontal, 8 s; vertical, 30 mV for V_m (membrane potential), 20 nA for I_c (voltage-clamp current) and 100 mV for L_{10} (the membrane potential of the presynaptic neurone). (From Wilson & Wachtel, 1978.)

iontophoretically applying ACh to the axon of the cell. The slow component of the ACh response was unaltered by a three-fold increase in the external potassium concentration, but was abolished in sodium-free solution or by lowering the temperature. Wilson & Wachtel (1978) concluded that prolonged cholinergic inhibition blocks a slow regenerative inward current that is responsible for the membrane potential oscillations seen in burster neurones (Wilson & Wachtel, 1974). Dopamine has a similar effect upon the slow regenerative current observed in the burster neurone R_{15} (Wilson & Wachtel, 1978). The slow voltage-dependent inward currents which are modulated by ACh and dopamine may involve an increase in conductance to calcium ions (Lewis & Wilson, 1983).

Co-transmitters

Histochemical and biochemical evidence is accumulating to indicate that a single neurone may contain more than one neurotransmitter. In some instances, the co-transmitters may be biochemically related, but in other instances they are not; thus peptides have been found co-localized with noradrenalin, 5-HT, dopamine or ACh, while some neurones show immunoreactivity to more than one peptide (Hökfelt *et al.* 1980).

Recently, Bishop & O'Shea (1982) demonstrated that an identified motor neurone (slow coxal depressor, Ds) of the cockroach, *Periplaneta americana*, contains the pentapeptide proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH). Since the excitatory neuromuscular transmitter in insects is generally considered to be L-glutamate (Usherwood, 1978), proctolin and glutamate presumably coexist in this motor neurone. Proctolin can be released from the terminals of the neurone either by

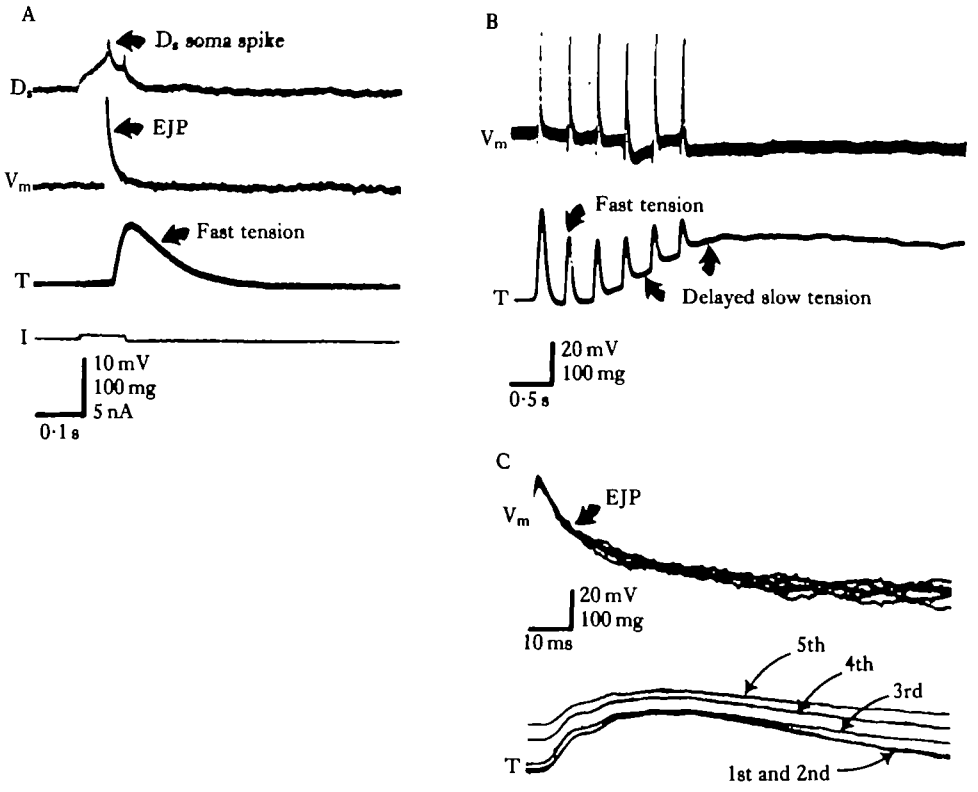

Fig. 11. Response of an insect muscle to activity in a peptide-containing motor neurone. (A) Upper trace, a single action potential in the slow coxal depressor motor neurone of the cockroach (D_s) evokes a brief excitatory junctional potential (EJP) in muscle fibres (V_m) and a transient tension response from the whole muscle (T). Trace I indicates the current injected into the motor neurone soma. (B) Repetitive motor neurone activity evokes a series of EJPs (trace V_m) without any sustained change in membrane potential. Each EJP is accompanied by a brief contraction, but muscle tension also shows a slow rise, which is not associated with any change in muscle fibre membrane potential (trace T). (C) During a burst of motor neurone spikes, there is a progressive slowing in the relaxation rate of rapid contractions (trace T), although the shape of successive EJPs is unaltered (trace V_m). The fast contractile responses may be produced by the action of glutamate, while the peptide proctolin may cause the slow component of contraction. (From Adams & O'Shea, 1983.)

intracellular stimulation of the motor neurone or by increasing the potassium concentration in the bathing solution; release was blocked by replacing external calcium with cobalt, which prevents synaptic release of neurotransmitter substances from nerve terminals (Adams & O'Shea, 1983). Tension and membrane potential recordings from the coxal depressor muscles innervated by this motor neurone show a brief excitatory junctional potential (EJP) and contractile response following single motor neurone action potentials. However, following trains of motor neurone spikes a second, slowly-developing contractile component appears. The relaxation phase following each of the brief tension responses also becomes progressively prolonged (Fig. 11). During the slow rise in tension there is no detectable change in either the muscle fibre membrane potential or input resistance; EJPs are also unaltered. When proctolin is applied to the muscle in low concentrations, it also causes a slow rise in muscle tension and prolongs the relaxation phase of the transient tension responses.

Adams & O'Shea (1983) have concluded that the transient tension and electrical responses observed in the coxal depressor muscles are produced by synaptically released glutamate, while the slow tension response is mediated by proctolin acting as a co-transmitter.

The action of proctolin on cockroach muscle has similarities with the effects of 5-HT on buccal muscle of *Aplysia* (Weiss, Cohen & Kupfermann, 1975, 1978; Kupfermann *et al.* 1979). When 5-HT is released from peripheral terminals of an identified serotonergic neurone, it potentiates contractions evoked by cholinergic motor neurone activity. Applied or synaptically released 5-HT has no detectable effect upon resting tension, membrane potential or input resistance of muscle fibres, although it does produce a modest increase in amplitude of EJPs. However, the enhancement of EJPs cannot account for the effects of 5-HT, as the time course of the enhancement in contractility is not necessarily the same as that for augmentation of EJPs. In addition, activation of the serotonergic neurone potentiates the contractile response to applied ACh. These observations indicate that 5-HT enhances contraction of *Aplysia* muscle by a postsynaptic mechanism which does not involve any electrical change in the muscle fibres. It has been suggested that cyclic AMP mediates a direct action of 5-HT upon excitation-contraction coupling (Weiss *et al.* 1978; Kupfermann *et al.* 1979). It is possible that similar mechanisms may be involved in the postsynaptic actions of octopamine on locust muscle (O'Shea & Evans, 1979) and of octopamine, 5-HT and proctolin on lobster muscle (Kravitz *et al.* 1980; Schwarz, Harris-Warrick, Glusman & Kravitz, 1980).

CONCLUDING REMARKS

The complexity of chemically mediated synaptic transmission provides a substrate for considerable flexibility; not only can different synapses have individual properties, but also the characteristics of any single synapse may depend upon its recent history of activity. This versatility enables quite diverse functional requirements to be met. Some synapses, like the vertebrate neuromuscular junction, may be required to transmit information rapidly with a large safety factor, while others may perform more subtle tasks over a longer time-scale.

Transmitter release will depend upon both its availability for release and upon the characteristics of the release process. Individual synapses may show differences in the rate at which released transmitter is replenished; this may become important at peptidergic synapses, since peptide neurotransmitters cannot be synthesized in nerve terminals. It may turn out, however, that peptides are released at junctions where the overall transmitter requirement does not fluctuate greatly or that their actions are sufficiently prolonged that short-term adjustments in supply are not required.

The sensitivity of transmitter release to changes in presynaptic membrane potential may differ considerably between synapses and will depend largely upon the types of ion channel in the membrane of the presynaptic terminal. It is, therefore, not surprising that changes in the resting potential or in the action potential amplitude may have different effects at different synapses. Transmitter release at some synapses is extremely sensitive to small shifts in membrane potential. This has important functional implications, since it indicates that a considerable amount of

integration can take place by means of local graded interactions restricted to limited portions of neurones.

The ability of neurotransmitters to operate voltage-dependent channels has a number of interesting consequences. Operation of such channels provides one mechanism for presynaptic inhibition. Calcium influx into presynaptic terminals may be influenced by direct modulation of calcium channels or may be altered indirectly as a result of an effect upon other channels that themselves alter the configuration of the action potential. If the modification in the calcium current of the action potential is widespread throughout a neurone, it may cause a change in the amount of transmitter released per impulse from spatially separated transmitter release sites. This may be possible, since synaptic activation of voltage-dependent channels can alter the configuration of the action potential recorded from the cell body of the neurone. Transmitter-operated voltage-sensitive channels provide the potential for extremely non-linear summation of different synaptic inputs; if a transmitter operates voltage-dependent channels which can only open at membrane potentials more positive than the normal resting potential, this transmitter would, by itself, be inoperative and would require some other input to 'enable' it. Conversely, this voltage-dependent response could be completely 'gated out' by any transmitter which hyperpolarized the membrane potential beyond the range in which channels operate. Transmitter actions upon voltage-dependent channels can also alter the pattern of activity in a neurone. For example, it has been suggested that the potassium M-current in sympathetic neurones may normally limit impulse frequency since it will generate an outward current when it is activated by depolarization. Synaptic inactivation of this potassium current will enhance excitability. Although the transmitter itself does not cause strong excitation, it may prolong excitation generated by another synaptic input. The ability of transmitters specifically to increase or decrease the conductances underlying 'burster' activity may be important in regulating the properties of 'oscillator' circuits. This type of synaptic action could turn a network on or off and may even switch it from one operating mode to another.

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