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CHAPTER EIGHT

INDIRECT MECHANISMS OF SYNAPTIC TRANSMISSION

The majority of neurotransmitters do not bind directly to ligand-activated ion channels, but rather to receptors that influence ion channels and pumps indirectly through membrane-associated or cytoplasmic second messengers. At some synapses, transmission occurs solely by such indirect mechanisms. Other synapses are modulated by indirect action.

Even at a synapse as reliable and simple as the skeletal neuromuscular junction, transmission can be modulated. Norepinephrine released from axons of the sympathetic nervous system causes a long-lasting increase in the amplitude and duration of the end plate potential, both by increasing the amount of transmitter released from motor axon terminals and by changing the properties of the muscle fiber membrane. In the sympathetic ganglion of the bullfrog two different transmitters—ACh and a peptide resembling luteinizing hormone-releasing hormone (LHRH)—modulate synapses. Both bind to receptors that cause voltage-activated M-type potassium channels to close. As a result, excitatory inputs that would normally evoke a single impulse produce instead trains of action potentials. At many synapses, indirectly acting transmitters affect channels that are not open in the resting cell. For example, norepinephrine inhibits a calcium-activated potassium channel on neurons in the rat hippocampus. This causes little or no change in the resting potential but enhances the response of the cells to excitatory inputs. Such changes in the efficacy of transmission at synapses are called neuromodulation.

The time course of the response to activation of an indirectly coupled receptor is much slower than the rapid kinetics of synaptic potentials produced by directly coupled, ligand-activated channels; the response can last for seconds, minutes, or hours rather than milliseconds. In addition, transmitters released from one axon terminal may diffuse to modulate activity in many target cells. In each cell specificity of action is determined by the nature and distribution of the transmitter receptors.

The responses to activation of indirectly coupled receptors are mediated by G proteins, so named because they bind guanosine diphosphate (GDP) and guanosine triphosphate (GTP). G proteins modify the activity of other receptor proteins, ion channels, or pumps. G proteins are trimers of three subunits, α, β, and γ. The α

subunits interact with receptor and effector proteins, whereas the $\beta\gamma$ complex acts primarily to anchor the α subunit to the membrane. GDP bound to the α subunit keeps the $\alpha\beta\gamma$ complex intact. When a G protein is activated by interaction with a transmitter receptor, GDP is replaced by GTP, and the α subunit dissociates from the $\beta\gamma$ complex. The free α subunit binds to and modulates the activity of its target—an enzyme, a channel, or a pump. G proteins are grouped into classes according to the targets recognized by the α subunit: G_t activates cyclic GMP phosphodiesterase, G_s stimulates adenylyl cyclase, G_i inhibits adenylyl cyclase, G_p activates phospholipase C, G_k activates potassium channels, and G_o acts on still other proteins. A particular G protein can activate more than one type of target, and the activity of a target protein is often modified through more than one G protein pathway.

Phosphorylation of serine and threonine residues by enzymes called protein kinases is a common mechanism by which the activity of receptors and ion channels is modified. Adenylyl cyclase, an enzyme whose activity is modulated by G_i and G_s, catalyzes the synthesis of cyclic AMP, which, in turn, increases the activity of cyclic AMP-dependent protein kinase. The activation of phospholipase C by G_p results in both the formation of diacylglycerol and an increase in intracellular calcium concentration, which together activate protein kinase C. In addition to its stimulatory effect on protein kinase C, increased intracellular calcium concentration also activates calcium–calmodulin-dependent protein kinase.

In a wide variety of cells, potassium and calcium channels are prime targets for indirect transmitter action. Changes in channel activation in axon terminals can modify calcium entry, and thereby transmitter release, both directly, through effects on calcium channels, and indirectly, by altering the duration of the action potential. Changes in calcium and potassium channels in postsynaptic cells can alter spontaneous activity and the response to synaptic inputs.

The mechanisms by which neurotransmitters produce effects in their target cells can be divided into two general categories: direct and indirect. Direct mechanisms, discussed in Chapter 7, are also referred to as "fast" or "channel-coupled." In such cases the postsynaptic receptor is itself a ligand-activated channel, which contains both the transmitter binding site and the ion channel opened by the transmitter as part of the same protein. Indirectly acting transmitters, on the other hand, bind to receptors that are not themselves ion channels, but which modify the activity of other receptor proteins, ion channels, or ion pumps so that the response of the cell is altered. Indirect mechanisms are also referred to as "slow" or "second messenger-linked." Many indirectly acting receptors produce their effects through interaction with GTP-binding, or G PROTEINS.[1]

[1]Dunlap, K., Holz, G. G. and Rane, S. G. 1987. *Trends Neurosci.* 10: 244–247.

At some synapses, such as those on smooth muscle of the gut, transmission may occur solely through indirectly coupled receptors. Alternatively, indirectly acting transmitters may influence the efficacy of transmission at synapses where other transmitters are released, a process referred to as NEUROMODULATION.

MODULATION OF SYNAPTIC TRANSMISSION

Neuromodulation at the neuromuscular junction

Before discussing the mechanisms by which indirectly coupled receptors produce their effects, it is useful to illustrate how indirect transmitter action can modify synaptic efficacy. It is somewhat ironic that one of the first synapses at which neuromodulation was described was the vertebrate skeletal neuromuscular junction. After all, the function of the neuromuscular junction, as described by Katz,[2]

> is to transfer impulses from the relatively very small motor nerve endings to the large muscle fiber and cause it to contract. At most of the myoneural junctions in vertebrate muscle, each nerve impulse is followed by a similar impulse in the muscle fibers. . . . Thus, the vertebrate myoneural junction serves a much simpler purpose than the central synapses of neurons . . . , where integration of converging signals takes place and where the effect of a single nerve impulse is generally well below the threshold of excitation of the effector cell. To put it somewhat crudely, the vertebrate nerve–muscle junction serves the purpose of a simple relay.

In spite of this apparent simplicity, direct cholinergic transmission at the neuromuscular junction is modulated in complex ways by several substances. For example, as early as 1923 it was shown that the adrenergic agents norepinephrine (noradrenaline), released from varicosities along sympathetic axons, and epinephrine (adrenaline), released into the circulation from the adrenal medulla, facilitated neuromuscular transmission.[3] For nearly half a century there was considerable confusion regarding the mechanism of this effect. Some experiments suggested a presynaptic action, others a change in the muscle fiber. Both results were found to be correct, but mediated by different receptors. Epinephrine and norepinephrine act on a variety of receptors, all of which are indirectly coupled to their effector proteins. Based on the relative potency of different agonists and antagonists, adrenergic receptors can be divided into five main classes: α_1, α_2, β_1, β_2, and β_3. The β_1 receptors predominate on cardiac tissue; β_2 and α_1 receptors are found primarily in smooth muscle, skeletal muscle, and liver cells; β_3 receptors are localized to adipose tissue; and α_2 receptors are commonly found on presynaptic nerve terminals and on postsynaptic cells in a variety of tissues.[4] At the skeletal neuromuscular junction, activation of α-adren-

[2]Katz, B. 1966. *Nerve, Muscle, and Synapse.* McGraw-Hill, New York.

[3]Orbeli, L. A. 1923. *Bull. Inst. Sci. Leshaft* 6: 194–197.

[4]Lefkowitz, R. J., Hoffman, B. B. and Taylor, P. 1990. *In* A. G. Gilman et al. (eds.). *Goodman and Gilman's Pharmacological Basis of Therapeutics,* 8th Ed. Pergamon Press, New York, pp. 84–121.

ergic receptors on the presynaptic nerve terminal was found to increase the number of quanta of transmitter released by an action potential. Stimulation of β-adrenergic receptors on the muscle fibers activates the Na–K pump (Chapter 3), causing hyperpolarization and a decrease in the resting membrane conductance (Figure 1).[5,6] Thus both pre- and postsynaptic adrenergic receptors contribute to the increase in the amplitude and duration of the synaptic potential.

Varicosities along sympathetic axons in skeletal muscle are not specifically juxtaposed to neuromuscular junctions. Sympathetic axons course randomly through muscle and release norepinephrine from varicosities scattered along their lengths (Figure 1A). Accordingly, norepinephrine must diffuse some distance from its site of release to reach receptors on the axon terminal and the muscle fiber. In addition, norepinephrine released from one varicosity may affect transmission at many junctions. Thus the specificity of norepinephrine's actions is achieved not by precise anatomical connections but by the nature and distribution of the receptors to which it binds. This is a common feature of neuromodulatory pathways.

Another general feature of neuromodulatory actions is that they have a prolonged time course. At the neuromuscular junction the effects of norepinephrine develop slowly over 15 to 20 minutes and decline slowly after norepinephrine is removed. This slow time course arises in two ways. First, a short delay of seconds is introduced by the time required for diffusion from the site of release to the site of action. Second, and more importantly, the indirect mechanisms by which α- and β-adrenergic receptors produce their effects involve changes in cell metabolism that have very slow kinetics compared with those of ligand-activated ion channels. Once set in motion these metabolic processes can continue long after the transmitter has diffused away from the receptors.

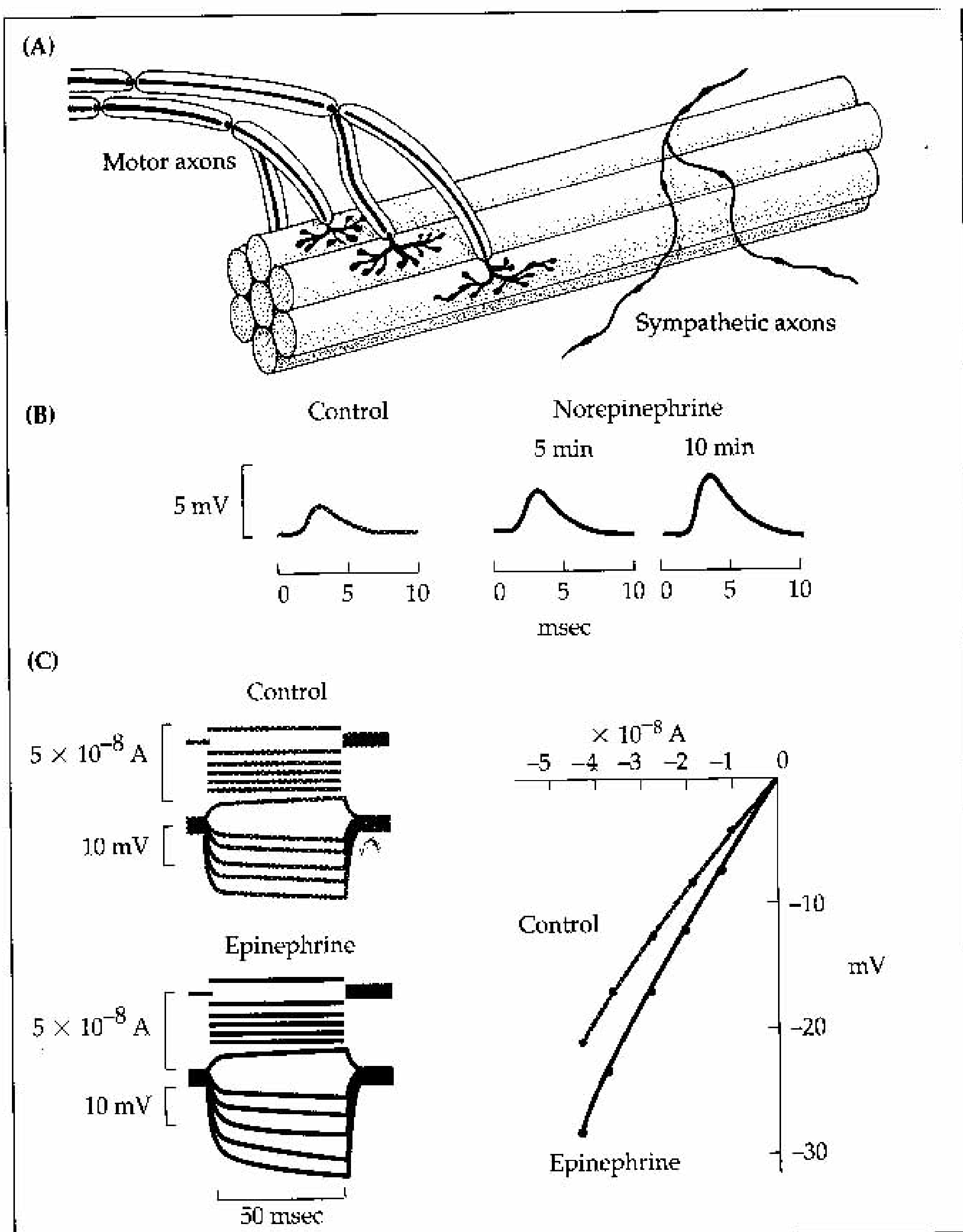
Neuromodulation in sympathetic ganglia

An example of how neuromodulatory effects can alter the pattern of signaling between neurons is provided by experiments of Libet, Nishi, Koketsu, Weight, and others, who described slow synaptic potentials with unusual properties in sympathetic ganglion cells of frogs and mammals. Stimulation of presynaptic inputs to the ganglion cells elicits three depolarizing potentials: a fast excitatory postsynaptic potential (epsp); a slow epsp, and a late slow epsp[7] (Figure 2A). With single presynaptic impulses, the slow epsp and late slow epsp are not evident; they are seen only after trains of action potentials at natural frequencies. Even with a train of impulses the slow and late slow epsps are not sufficient by themselves to bring the cell to threshold. However, they do alter the response of the cell to subsequent stimuli, as we shall see.

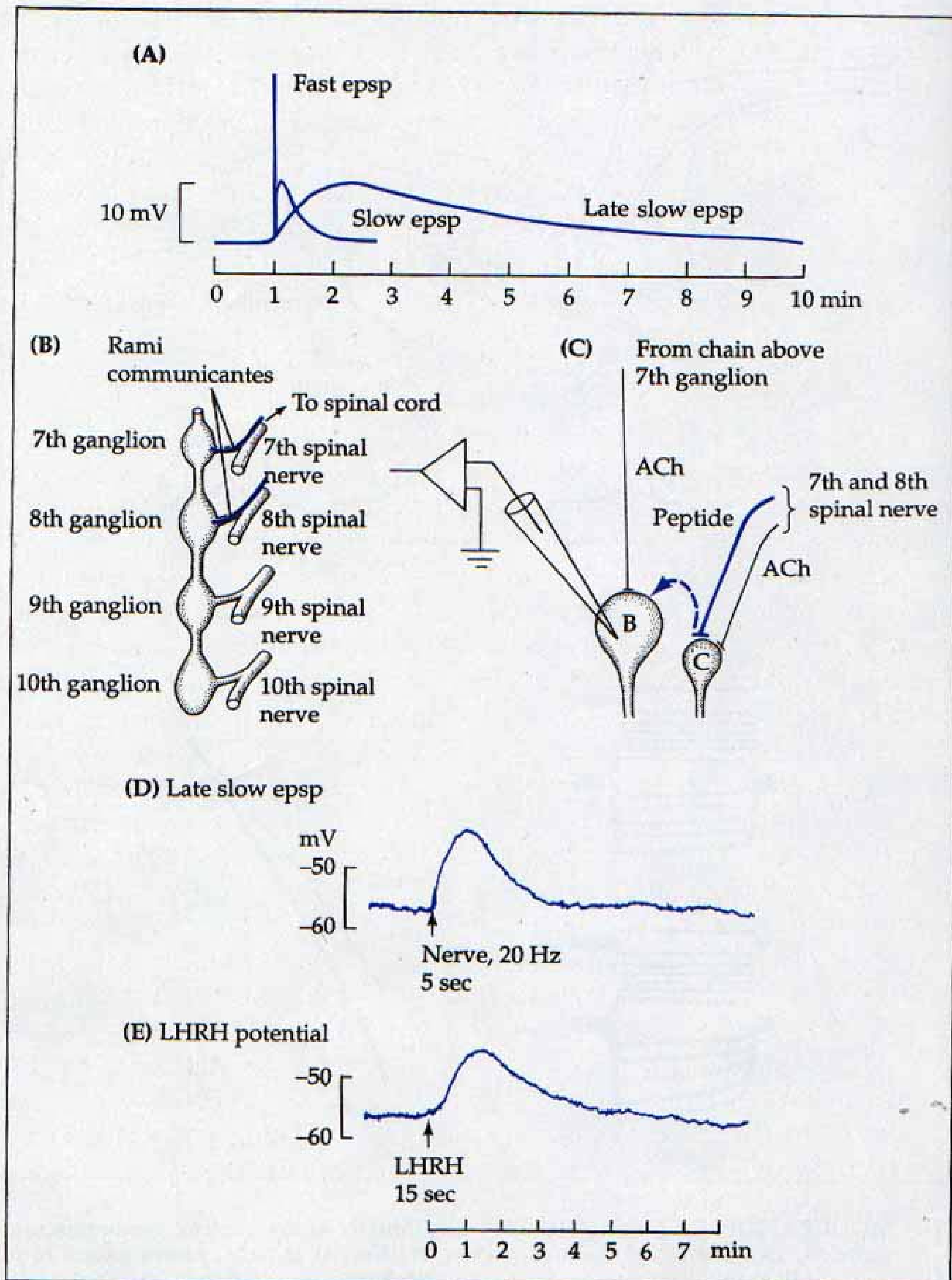
[5]Kuba, K. 1970. *J. Physiol.* 211: 551–570.

[6]Clausen, T. and Flatman, J. A. 1977. *J. Physiol.* 270: 383–414.

[7]Jan, Y. N., Jan, L. Y. and Kuffler, S. W. 1979. *Proc. Natl. Acad. Sci. USA* 76: 1501–1505.



1 **MODULATION OF SYNAPTIC TRANSMISSION at the skeletal neuromuscular junction. (A) Sketch of the innervation of skeletal muscle. Motor axons form discrete, highly specialized synapses where postsynaptic receptors and presynaptic release sites are precisely juxtaposed. Sympathetic axons course throughout the muscle. Norepinephrine, released from varicosities scattered along their length, diffuses to reach receptors on the muscle and axon terminal. (B) Addition of 10^{-6} g/ml norepinephrine causes an increase in the amplitude of the synaptic potential evoked by nerve stimulation; this effect was blocked by specific antagonists of α-adrenergic receptors. The increase is slow to develop and is due to an increase in the number of quanta released. (Intracellular recordings from a muscle bathed in a solution containing 10^{-6} g/ml *d*-tubocurare so that the synaptic potentials would be subthreshold.) (C) Addition of 5×10^{-6} g/ml epinephrine causes a decrease in resting membrane conductance. Small current pulses were passed through an intracellular microelectrode to measure the conductance of the muscle fiber membrane. Ten minutes after epinephrine was added, the conductance of the membrane was decreased compared with control. This effect was blocked by β-adrenergic receptor antagonists. (After Kuba, 1970.)**



2 SYNAPTIC POTENTIALS IN SYMPATHETIC GANGLION CELLS in a bullfrog. **(A)** The fast, slow, and late slow epsp's evoked in B ganglion cells. A single stimulus to the preganglionic inputs evokes a large fast epsp; trains of stimuli (10/sec for 5 sec) are required to elicit the slow and late slow synaptic potentials. **(B)** Diagram of sympathetic ganglia 7, 8, 9, and 10. Nerve cells receive inputs from the spinal cord via rami communicantes, and also from the chain above. **(C)** The larger B cells in caudal ganglia (9 and 10) receive cholinergic input from spinal nerves rostral to ganglion 7 and receive peptide input indirectly from spinal nerves 7 and 8 via synapses onto neighboring C cells. Accordingly, the different inputs can be stimulated selectively. **(D)** The late slow excitatory potential evoked by stimulating spinal nerves 7 and 8 (20/sec for 5 sec). The depolarization lasts for several minutes. **(E)** Application of LHRH to the cell in (D) by pressure from a micropipette. The peptide mimics the action of the naturally released transmitter. (After Kuffler, 1980.)

Both the fast epsp and the slow epsp are evoked by the release of acetylcholine (ACh) from the presynaptic nerve terminals. The fast epsp results from activation of nicotinic ACh receptors. The resulting depolarization usually elicits a single action potential in the ganglion cell. The slow epsp is due to the binding of acetylcholine to muscarinic ACh receptors. Kuffler and his colleagues found that the late slow epsp in the sympathetic ganglion of the frog could be mimicked by application of the peptide luteinizing hormone-releasing hormone (LHRH)[7–10] (Figures 2D and 2E). (LHRH is a hormone released into the local circulation by neurons in the hypothalamus. It diffuses to the anterior pituitary gland and causes it to secrete luteinizing hormone, an essential hormone involved in the ovarian cycle and in the secretion of testosterone.) The dose required (1 μM or more) seemed at first too high for LHRH to be a serious transmitter candidate. With this as a clue, analogues of LHRH were tested and some were indeed found to be 100 times more potent than the hormone itself. A variety of other tests demonstrated that the late slow epsp was due to the release of an LHRH-like peptide. However, no nerve terminals containing an LHRH-like peptide were found juxtaposed to the B cells from which these recordings were made. The peptide reaches B cells by diffusion from synapses made on smaller, neighboring C cells (Figure 2C).[10] Thus transmitter liberated by a particular nerve terminal may act on more than one postsynaptic cell.

The cells of autonomic ganglia are particularly favorable for electrophysiological investigation of the mechanism of such slow neuromodulatory effects. The B cells are relatively large and nearly spherical, with no dendrites, and synaptic contacts are made directly onto the cell body. Voltage clamp studies of B cells by Adams, D. Brown, and their colleagues identified a current carried by a particular type of potassium channel that was modified by activation of muscarinic receptors, the so-called M (for muscarinic) current.[11] These M potassium channels are voltage-activated and have a threshold for activation that is near the normal resting potential. Accordingly, some of the channels are open at rest and provide a major contribution to the resting potassium conductance. Activation of muscarinic ACh receptors closes the M potassium channels (Figure 3A).

What are the consequences of the closure of potassium channels? The resting influx of sodium ions is no longer balanced by potassium efflux, so the cell depolarizes, producing the slow epsp. With trains of stimuli at natural frequencies, the depolarization produced by the slow epsp, about 10 mV, is not sufficient to bring the cell to threshold. Moreover, the change in membrane potential on its own has little effect on the response of the cell to the much larger fast epsp (Figure 3B).[12]

[8] Kuffler, S. W. 1980. *J. Exp. Biol.* 89: 257–286.

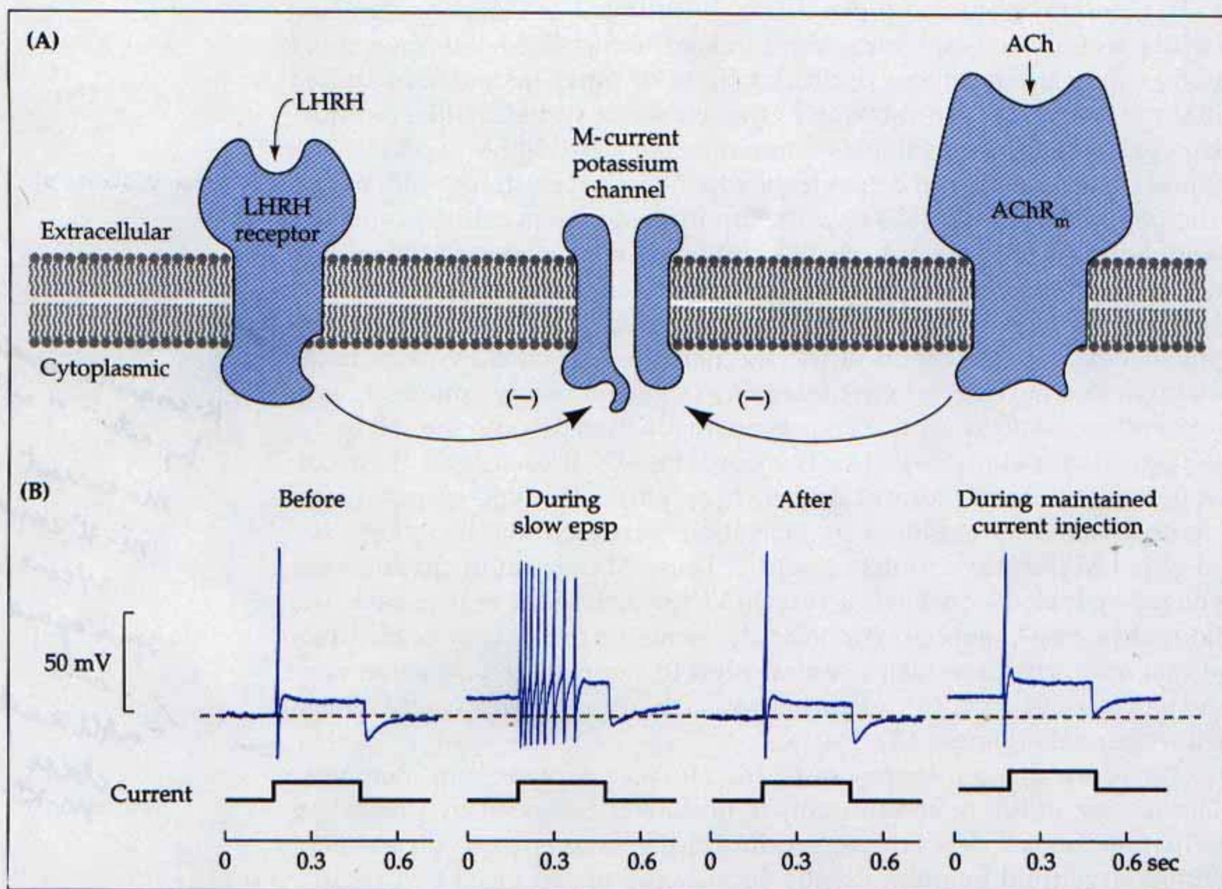
[9] Jan, Y. N., Jan, L. Y. and Kuffler, S. W. 1980. *Proc. Natl. Acad. Sci. USA* 77: 5008–5012.

[10] Jan, Y. N. et al. 1983. *Cold Spring Harbor Symp. Quant. Biol.* 48: 363–374.

[11] Adams, P. R., Brown, D. A. and Constanti, A. 1982. *J. Physiol.* 330: 537–572.

[12] Adams, P. R., Brown, D. A. and Constanti, A. 1982. *J. Physiol.* 332: 223–262.

When M-current channels are closed, however, the response of the B cell to the fast epsp is dramatically enhanced. The decrease in membrane conductance increases the amplitude of excitatory synaptic potentials (see Chapter 7), as described above for norepinephrine at the neuromuscular junction. This effect is amplified during depolarizing synaptic potentials in the following way. M channels are normally activated by depolarization and repolarize the cell, cutting short excitatory synaptic potentials. When M-current channels are kept closed by activation of muscarinic receptors, the increase in potassium conductance is prevented and depolarizations are not curtailed. The result is that the fast epsp elicited by stimulation of the preganglionic nerve, which nor-



3 INHIBITION OF POTASSIUM CURRENTS IN SYMPATHETIC GANGLION CELLS modulates response to presynaptic stimulation. (A) Binding of ACh to muscarinic receptors (AChR$_m$) and binding of LHRH to its receptor both inhibit M-current potassium channels. (B) The effect of the decrease in the M current during the slow epsp is to increase the excitability of the B cell. Depolarizing current pulses before and after a slow epsp produce a single action potential. During the slow epsp, the same current pulse elicits a burst of action potentials. Depolarizing the B cell to the same extent as occurs during the slow epsp by injecting a maintained current has no such effect on the responsiveness of the cell. (After Jones and Adams, 1987.)

mally gives rise to one or two impulses, now becomes prolonged and evokes a train of action potentials in the B cell (Figure 3B).[13] Thus the slow epsp modifies the pattern of transmission across the ganglionic synapses. It is tempting to speculate about the role of such mechanisms in the regulation of functions that are controlled by the autonomic nervous system, such as blood pressure, gastrointestinal motility, and glandular secretion, as well as in disorders such as hypertension, glaucoma, or gastrointestinal ulcers that might result from autonomic dysfunction.

The mechanism of the peptide-evoked late slow epsp is the same as that of the slow ACh-mediated epsp; M-current potassium channels are closed (Figure 3A).[14,15] Moreover, a saturating response to prolonged application of LHRH completely occludes the response to activation of muscarinic receptors, and vice versa. Thus, although the receptors for ACh and the LHRH-like peptide are clearly distinct, they act through a common final pathway. The only difference is the time course of their effects, which appears to be determined by the time course of transmitter release, diffusion, and inactivation.

Neuromodulation of hippocampal pyramidal neurons

As the preceding discussion suggests, indirectly acting transmitters can modulate signaling without producing any change in the resting potential of the target cell. An example comes from studies of the effects of norepinephrine on neurons in the hippocampus by Nicoll and his colleagues. This region of the vertebrate central nervous system has been the focus of extensive investigation because of its relatively simple architecture and its importance for short-term memory (see Chapter 10).[16] Hippocampal pyramidal cells, like many other vertebrate neurons, have a slow calcium-activated potassium current.[13] The influx of calcium during an action potential causes these potassium channels to open, which in turn causes a prolonged hyperpolarization, the so-called slow after-hyperpolarization (AHP). Norepinephrine blocks this current[17] (Figure 4). Since few of these channels are open at rest, application of norepinephrine to pyramidal cells produces little or no change in resting potential. However, when the slow calcium-activated potassium current is inhibited by addition of norepinephrine, excitatory inputs that might normally produce only a few action potentials before being cut short by the after-hyperpolarization now evoke a sustained train of impulses (Figure 4B). Like the M-current channels in frog sympathetic ganglion cells, the slow calcium-activated potassium channels in hippocampal pyramidal cells are modulated by several neurotransmitters, each acting through its own receptor.[16]

[13]Jones, S. W. and Adams, P. R. 1987. *In* L. K. Kaczmarek and I. B. Levitan (eds.). *Neuromodulation: The Biochemical Control of Neuronal Excitability*. Oxford University Press, New York, pp. 159–186.

[14]Adams, P. R. and Brown, D. A. 1980. *Br. J. Pharmacol.* 68: 353–355.

[15]Kuffler, S. W. and Sejnowski, T. J. 1983. *J. Physiol.* 341: 257–278.

[16]Nicoll, R. A. 1988. *Science* 241: 545–551.

[17]Madison, D. V. and Nicoll, R. A. 1986. *J. Physiol.* 372: 221–244.