Synaptic Transmission: Long-Lasting Potentiation by a Postsynaptic Mechanism

Abstract. *Slow decreases of ionic conductance across neuronal cell membranes, which generate slow synaptic potentials, can increase the effectiveness of synaptic transmission. Slow conductance decreases of sufficient magnitude increase the amplitude of monosynaptic fast excitatory postsynaptic potentials in B cells of the bullfrog sympathetic ganglion. By this postsynaptic mechanism, activation of one synaptic pathway can cause an increase in transmission, lasting several minutes, across another synapse. This may provide an important mechanism for synaptic integration and control of neuronal interaction.*

An understanding of the mechanisms of synaptic transmission and their regulation may be important in the elucidation of the cellular mechanisms underlying complex nervous functions. The postsynaptic mechanisms by which excitatory postsynaptic potentials (EPSP's) are generated and combine might provide a locus for control of synaptic function. However, the vast majority of known postsynaptic electrical responses are of short duration (tens of milliseconds). Thus, postsynaptic interactions of such electrical responses are necessarily brief and require a high degree of synchrony of convergent inputs. These interactions, therefore, do not provide lasting modification of responsiveness. In addition, when fast synaptic potentials combine, the result is at most their algebraic sum, and often is less (*1*). We report here a postsynaptic mechanism by which activation of one synaptic pathway can increase transmission in a known monosynaptic second pathway. Further, component postsynaptic responses combine to generate potential changes that are larger than their algebraic sum. This effect can last several minutes, and thus is unusually long for known integrative mechanisms in the vertebrate nervous system.

At most synapses, the postsynaptic potentials (PSP's) are generated by increases of ionic conductance across the nerve cell membrane (*2*). However, it has been shown that slow synaptic potentials in several neuron types can be generated by decreases in ionic conductance (*3*). Decreases of postsynaptic membrane conductance have been detected during synaptically and pharmacologically generated potential changes in mammalian cortex (*4*) and cerebellum (*5*), in vertebrate sympathetic ganglia (*6*), and in neurons of invertebrates (*7, 8*). In a prediction based on Ohm's law, it was suggested that the decrease in resting membrane conductance (increase in resistance) should increase the amplitude of conductance-increase PSP's by increasing the voltage drop generated by a given synaptic current flow (*9*). To investigate the possible functional role of conductance decreases in the control of synaptic function, we recorded intracellularly from cells of the excised tenth paravertebral sympathetic ganglion of the bullfrog *Rana catesbeiana*.

Stimulation of preganglionic afferents evokes both conductance-increase and conductance-decrease PSP's in sympathetic ganglion neurons. Stimuli applied to preganglionic B fibers (*10*) in the sympathetic chain produce nicotinic cholinergic fast EPSP's, which are generated by increased ionic conductance (*11*). This pathway is known to be monosynaptic (*12*) and in all but a few instances a single preganglionic B fiber innervates each B cell. In these cells, stimulation of B afferents also produces a muscarinic cholinergic slow EPSP, which is generated by a decreased ionic conductance (*6*). Since the tetanic stimulation used to evoke the slow EPSP affects subsequent transmitter release from B-fiber terminals, and thus affects the amplitude of fast EPSP's, iontophoretic application of the muscarinic agonist methacholine (MCh) was used to mimic the slow EPSP (Fig. 1C).

The MCh-induced conductance change closely paralleled that induced by repetitive preganglionic B-fiber stimulation (*13*). The effect of a conductance decrease on synaptic transmission was therefore studied first by observing the effect of iontophoretically administered MCh on fast EPSP's. The effect of MCh on fast EPSP's showed a strong dependence on the magnitude of the conductance change, which varied from cell to cell. Fast EPSP amplitude increased with large decreases in conductance and decreased with small conductance decreases. Decreases of fast EPSP amplitude are presumably due to the depolarization of the membrane during the MCh response (*14*). Figure 1 shows the increase of fast EPSP amplitude caused by the iontophoretic administration of MCh. In Fig. 1A, fast EPSP's are shown before and during the MCh response. Both the amplitude and duration of the fast EPSP's are increased as the result of MCh administration. The change in amplitude and shape of the fast EPSP are shown particularly well in the superimposed tracings of the fast EPSP's before and during the MCh response (Fig. 1B). The average peak amplitudes of the fast EPSP's changed from 5.6 ± 1.3 mv ($\pm$ standard deviation) in the control to 7.9 ± 2.7 mv ($N = 20$; $P < .005$) during the MCh response. The peak EPSP amplitudes were also averaged excluding EPSP's with obvious local responses; the means of 5.6 ± 1.3 mv (control) and 6.6 ± 1.8 mv (during MCh) are significantly different ($N = 20$; $P < .05$). The half-width of the EPSP's increased from an average of 21.8 ± 1.6 msec in the control to 28.3 ± 3.7 msec ($N = 20$; $P < .001$) during the response. The lengthening of the fast EPSP may be due to an increase of the resistance capacitance time constant of the membrane caused by the decreased membrane conductance (increased resistance) (*15*). In addition, these enlarged EPSP's often activated local responses and action potential generation (*16*). Furthermore, the peak depolarization reached by a fast EPSP arriving during the conductance-decrease depolarization was greater than the sum of the separately evoked fast and slow depolarizations.

Two types of experiments separate the effects of membrane depolarization on fast EPSP amplitude from the effect of decreased membrane conductance. First, as reported previously, electrical depolarization of the membrane decreased, but did not increase, the fast EPSP amplitude (*11*). Second, membrane potential was held at resting level during the MCh response. Figure 1D illustrates the fast EPSP amplitude distribution prior to and during the MCh response, in the absence of a membrane potential change. The average fast EPSP amplitude increased from 2.7 mv in the control to 3.3 mv during the MCh response ($N = 20$). The increase in the average fast EPSP amplitude was statistically significant ($P < .025$). Note also the increase in the number of larger amplitude EPSP's, for example, the number of EPSP's greater than 3.25 mv increased from three in the control to ten during the MCh response. Since EPSP's greater than a certain amplitude trigger action potential generation, such an increase in the number of large EPSP's can have a very significant effect on transmission of impulses at a synapse. In this and in many other experiments, fast EPSP amplitude was reduced with *d*-tubocura-

rine (*d*TC), 1 to 25 μg/ml, to prevent action potential generation during the MCh response. Experiments in which *d*TC was used produced results no different from those in which *d*TC was not used.

It is important to know whether such a postsynaptic potentiation of synaptic transmission can be elicited by stimulation of physiological pathways. To test this possibility, we utilized a separate preganglionic pathway to generate conductance decreases. Repetitive stimulation of the eighth spinal nerve produces in B cells a slow depolarization lasting many minutes, the late slow EPSP (*17*).

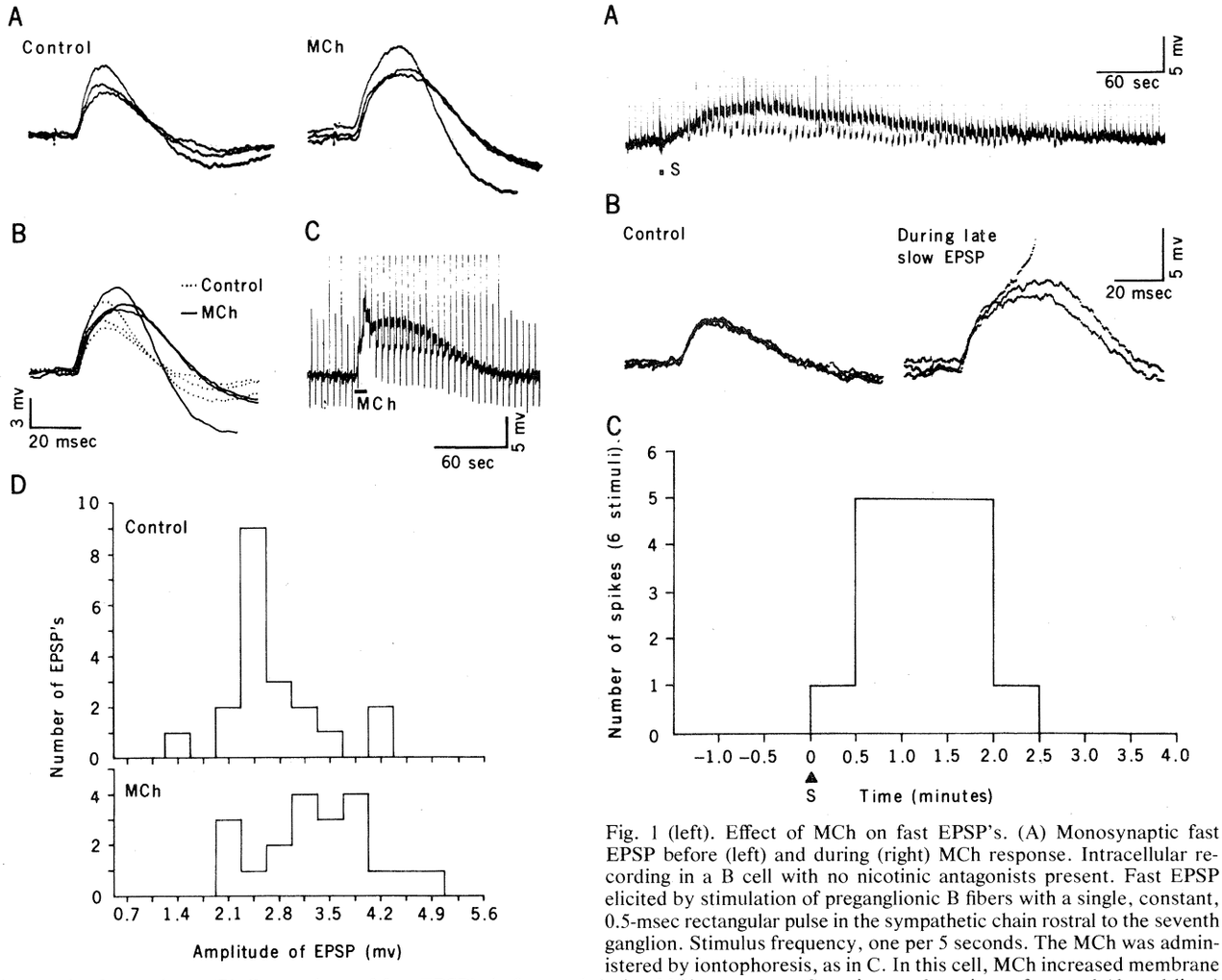

Fig. 1 (left). Effect of MCh on fast EPSP's. (A) Monosynaptic fast EPSP before (left) and during (right) MCh response. Intracellular recording in a B cell with no nicotinic antagonists present. Fast EPSP elicited by stimulation of preganglionic B fibers with a single, constant, 0.5-msec rectangular pulse in the sympathetic chain rostral to the seventh ganglion. Stimulus frequency, one per 5 seconds. The MCh was administered by iontophoresis, as in C. In this cell, MCh increased membrane resistance by 8 megohms. (B) Comparison of fast EPSP's before and during MCh responses. Superimposed tracings of control (dotted lines) and potentiated (solid lines) EPSP's from A. Calibration in B also applies to A. (C) Response of B cell to MCh administered by iontophoresis. Time of administration indicated by MCh. Changes in membrane resistance tested by current pulses of −0.15 na and 2 seconds in duration applied intracellularly every 5 seconds by way of a Wheatstone bridge. The bridge was balanced before drug application such that membrane potential deflections produced by current pulses did not appear as deflections from the recorded baseline. At the peak of the slow muscarinic depolarization input resistance of the membrane increased by 23 megohms. During the period of MCh iontophoresis, there was a decrease in membrane resistance. A brief nicotinic increase in membrane conductance, not exceeding the duration of drug application, was frequently observed during the iontophoresis of MCh; it was antagonized by curare (*d*TC) and had the electrophysiological properties of the nicotinic fast EPSP. (D) Histograms of the effect of MCh on fast EPSP amplitudes. Control and experimental values were compiled from four MCh applications. Top: amplitude distribution of fast EPSP's for 25 seconds prior to administration of MCh. Bottom: amplitude distribution of fast EPSP during MCh response with membrane potential held at resting level. Values of EPSP amplitude were taken for 25 seconds beginning 20 seconds after the start of iontophoretic MCh administration. The MCh was administered for 10 seconds, as in C. Fast EPSP's were evoked one per 5 seconds. To prevent the firing of action potentials by synaptic excitation, fast EPSP's were reduced in amplitude by 5 μg/ml of *d*TC. Records in C and D from the same neuron; A and B are from a different cell than C and D. Records in A and B are from photographs of an oscilloscope; records in C are from a rectilinear pen recorder (Brush model 280). Standard electrophysiological techniques were used for intracellular recording (*6*). Fig. 2 (right). Effect of late slow EPSP on fast EPSP's. (A) Late slow EPSP (elicited by repetitive stimulation of the eighth spinal nerve at a frequency of 50 hz for 2 seconds) with conductance decrease. The period of stimulation is indicated by *S*. Intracellular recording in a B cell. Current pulses of −0.15 na and 2 seconds duration were applied every 5 seconds as in Fig. 1C. Membrane resistance increased by 19.5 megohms during the late slow EPSP. (B) Monosynaptic fast EPSP's elicited by stimulation of sympathetic chain once per 5 seconds (see Fig. 1). Left: records of fast EPSP's prior to stimulation of the eighth nerve. Right: records of fast EPSP's during late slow EPSP. Note the increased fast EPSP amplitude and duration, as well as the initiation of an action potential. Stimulation of eighth nerve as in A; records from a different B cell. Resistance increase during late slow EPSP, 6.5 megohms. Concentration of *d*TC, 25 μg/ml. (C) Effect of late slow EPSP on spike generation by fast EPSP's. Same test as in B with fast EPSP elicited at a frequency of one per 5 seconds. The graph divides the experimental period into 30-second segments. During each segment, six fast EPSP's were elicited. During the 4.5-minute control period, no fast EPSP's fired spikes. After stimulation of the eighth nerve (labeled *S*), enlarged fast EPSP's began to fire spikes. Spike generation ceased after about 2.5 minutes, although fast EPSP's remained enlarged for the duration of the late slow EPSP. The intracellular experiments reported here were corroborated by separate experiments in which we recorded from postganglionic axons utilizing suction electrodes or the sucrose gap technique. In partially curarized ganglia, transmission of preganglionic B volleys through the ganglion was increased by conditioning stimulation of the eighth nerve (*20*).

Membrane conductance decreased during the late slow EPSP (Fig. 2A) and, as with MCh responses, the magnitude of the conductance decrease varied from cell to cell. The amplitude and duration of the fast EPSP increased during the late slow EPSP (Fig. 2B), and the increased amplitude fast EPSP's often initiated action potential generation (Fig. 2B). In the experiment shown in Fig. 2C, prior to eighth nerve stimulation, no fast EPSP's fired spikes, but after stimulation the fast EPSP's generated spikes for about 2.5 minutes. At the peak of the response, five out of six fast EPSP's fired action potentials, which is a clear demonstration of the facilitation of transmission in one synaptic pathway by activation of another.

In the preceding experiments, when the pharmacological or physiological stimuli produced large decreases in postsynaptic membrane conductance, they increased fast EPSP size. This suggests that the potentiation of synaptic transmission is caused by changes in postsynaptic membrane properties (*18*). Our findings indicate that different types of depolarizations have different consequences for neuronal interaction. That is, conductance increases (*19*) and electrical depolarization of the membrane (*11*) decrease synaptic potentials generated by a given excitatory input, whereas conductance decreases potentiate them. By augmenting, rather than diminishing, a synaptic input, a conductance decrease makes that input more effective in the generation of action potentials.

This control of the efficacy of synaptic transmission may be important in the function of neurons of the central nervous system. Central neurons receive an abundance of converging inputs, and several types of central neurons have conductance-decrease responses (*4, 5*). It is thus possible that increasing transmission in a synaptic pathway by a conductance-decrease response may have a function in information processing in the central nervous system. A synaptically evoked conductance decrease has been implicated in a behavioral response in the marine mollusc *Aplysia* (*8*). Further, lasting modification of responsiveness to a synaptic input might be a mechanism for storage of information at the cellular level. Long-term control of postsynaptic membrane properties might provide such a modification of responsiveness.

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References and Notes

1. R. E. Burke, *J. Neurophysiol.* **30**, 1114 (1967). At the neuromuscular junction, however, a nonlinearity in receptor sensitivity can produce a more than arithmetical summation of responses to low concentrations of endogenous or exogenous transmitter [B. Katz and S. Thesleff, *J. Physiol. (London)* **138**, 63 (1957); K. Magleby and D. Terrar, *ibid.* **244**, 467 (1975); H. Hartzel, S. Kuffler, D. Yoshikami, *ibid.* **251**, 427 (1975)].
2. B. Katz, *Nerve, Muscle, and Synapse* (McGraw-Hill, New York, 1966), pp. 146–148.
3. F. Weight, in *Synaptic Transmission and Neuronal Interaction*, M. V. L. Bennett, Ed. (Raven, New York, 1974), p. 141.
4. K. Krnjevic, R. Dumain, L. Renaud, *J. Physiol. (London)* **215**, 247 (1971).
5. G. Siggins, A. Oliver, B. Hoffer, F. Bloom, *Science* **171**, 192 (1971).
6. F. Weight and J. Votava, *ibid.* **170**, 755 (1970).
7. H. M. Gershenfeld and D. Paupardin-Tritsch, *J. Physiol. (London)* **243**, 457 (1974); J.-S. Kehoe, *ibid.* **244**, 23P (1975).
8. T. Carew and E. Kandel, *Science* **192**, 150 (1976).
9. F. Weight, in *The Neurosciences, Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1974), p. 929.
10. B. Libet, S. Chichibu, T. Tosaka, *J. Neurophysiol.* **31**, 383 (1968).
11. S. Nishi and K. Koketsu, *J. Cell. Comp. Physiol.* **55**, 15 (1960).
12. S. E. Johnson, *J. Comp. Neurol.* **29**, 385 (1918); C. C. Hunt and P. G. Nelson, *J. Physiol. (London)* **177**, 1 (1965).
13. Larger conductance decreases were measured in fall and spring than in winter.
14. Different cells produce depolarizations with different size resistance changes. When a large depolarization accompanies a small resistance change, the depressing effect of depolarization on fast EPSP size presumably overcomes the potentiating effect of the resistance increase, resulting in a net decrease of fast EPSP size. For fast EPSP's in the 2 to 4 mv range, increases of amplitude were observed for MCh-induced resistance increases (conductance decreases) of greater than 8 to 10 megohms and decreases in amplitude were observed for resistance increases of 4 megohms or less. With larger fast EPSP's (6 to 8 mv) potentiation could occasionally be observed even for small conductance decreases, perhaps because of activation of local responses. B. L. Ginsborg [*J. Pharmacol. Exp. Ther.* **150**, 217 (1965)] reported that MCh added to Ringers solution bathing the whole ganglion depressed synaptic transmission in these cells. Desensitization of receptors, depression of transmitter release, depolarization of the membrane, and small conductance changes are possible explanations for these findings.
15. Voltage-clamp analysis shows that decay of muscle end-plate potentials is determined by membrane time constant [A. Takeuchi and N. Takeuchi, *J. Neurophysiol.* **22**, 395 (1959)]. Fast EPSP's in ganglion cells of *Rana pipiens* are reported to decay slightly more slowly than electronic potentials generated by square current pulses (*11*).
16. The increased amplitude and duration of the fast EPSP may activate voltage- and time-dependent sodium conductances that may in turn contribute further to the increased duration of the EPSP. Activation of Na^+ conductances might also be triggered by a decrease in voltage-threshold caused by conductance decreases [see M. V. L. Bennett, B. Hille, S. Obara, *J. Neurophysiol.* **33**, 585 (1970)].
17. S. Nishi and K. Koketsu, *ibid.* **31**, 109 (1968).
18. Other possible mechanisms seem unlikely for several reasons. First, the potentiation of synaptic transmission cannot be mediated by interneurons because the test pathway is known to be monosynaptic. Second, changes of fast EPSP size depend on the magnitude of the slow conductance change. This argues strongly against the importance of presynaptic effects. In addition, there is no evidence of synaptic endings on synaptic terminals (H. Weitsen and F. Weight, *Brain Res.*, in press). Third, cooperativity in postsynaptic receptor activation seems unlikely, since the late slow EPSP is noncholinergic (*17*).
19. R. E. Burke, *J. Neurophysiol.* **30**, 1114 (1967); W. Rall, in *Neural Theory and Modeling*, R. Reiss, Ed. (Stanford Univ. Press, Palo Alto, Calif., 1964).
20. J. Schulman and P. Smith, unpublished observations.
21. We thank N. A. Busis, G. R. Siggins, and P. A. Smith for helpful discussions and J. Nash for assistance with computer analysis of the data.

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Mesenchyme-Dependent Morphogenesis and Epithelium-Specific Cytodifferentiation in Mouse Mammary Gland

Abstract. *Isografts of heterotypic recombinants of embryonic mammary epithelium with salivary mesenchyme undergo development morphogenetically resembling that of salivary gland. However, cytodifferentiation of the epithelium is like that of mammary gland. In lactating hosts these isografts respond to endogenous hormonal stimulation and synthesize a milk protein, α-lactalbumin.*

Many studies have shown that interactions between epithelium and stroma are required for organogenesis and, ultimately, cytodifferentiation to proceed (*1–4*).

To clarify the respective roles of epithelium and stroma in normal morphogenesis and cytodifferentiation, investigations of heterotypic recombinations offer a useful approach. Much has already been done in this area (*2*). Previous studies have tended to support the general principle that mesenchyme largely controls development of form (morphogenesis), while epithelium is the determinant of the type of cytodifferentiation to be expressed. To exemplify, in an early study of feather regeneration Wang (*5*) found

Table 1. Development of 16-day mammary gland epithelia combined with mammary and salivary mesenchyme, 3 weeks after transplantation under the kidney capsule in 4-week-old syngeneic female mice.

Source of mesenchyme	No. of explants	No. of grafts recovered	Developmental response of mammary epithelium
Renal stroma	20	4 (20%)	Degeneration
Mammary (16-day)	18	11 (61%)	Mammary gland-like
Salivary (14-day)	24	18 (75%)	Salivary gland-like