In contrast to the binding of zinc to large-molecular-weight fractions in cow's milk, we have demonstrated that human milk contains a small ligand that binds a large proportion of the total zinc. Analysis of human colostrum indicated that the low-molecular-weight zinc-binding ligand was present in greater quantities than in milk obtained later in lactation.

Almost 40 years ago, Brandt (*2*) suggested that the symptoms of AE resulted from deficiency of a nutrient in breast milk, which children with this condition required but could not obtain from other food sources because of a gastrointestinal disorder. Other investigators, however, believed that the disease was caused by infection of the gastrointestinal tract (*8*), and Dillaha *et al.* (*9*) treated an AE patient with diiodohydroxyquinoline for a yeast infection. The drug controlled the symptoms of AE and subsequently became the treatment of choice although its mechanism of action is still unknown (*4, 10, 11*). Moynahan (*12*) has proposed that in the intestine of AE patients there is a missing or defective enzyme which normally hydrolyzes a small peptide, a breakdown product of all dietary proteins except human milk (*11, 12*). According to this hypothesis the noxious oligopeptide chelates dietary zinc, reducing its availability, while the therapeutic action of diiodohydroxyquinoline results from a greater affinity for the peptide than for zinc, thereby freeing the metal for the metabolic needs of the host (*11*).

A simpler explanation not requiring the presence of a noxious factor is that the metabolic lesion in AE patients is a defect in the normal intestinal mechanism of zinc absorption. Lombech *et al.* (*13*) have reported that a 5-year-old male AE patient absorbed only 15 percent of a tracer dose of ^{65}Zn (control, 58 to 77 percent), while another 5-year-old male AE patient fed human milk absorbed 45 percent of the tracer dose.

In our study, the small-molecular-weight binding ligand isolated from human milk may enhance absorption of zinc in AE patients. It is possible that this zinc-binding ligand protects zinc from chelation with other dietary components thereby increasing the absorption of zinc, or that it actually transports zinc across the intestine prior to the development of specific mechanisms for intestinal absorption of the element. Thus, feeding either human milk or readily available forms of zinc could improve a defective zinc-absorption system.

It is not inconceivable that more or less species-specific binding ligands for a number of nutrients could exist in various milks. Such a mechanism to aid in the absorption of essential nutrients in newborn infants would be of obvious advantage for survival of the species. Future investigations of the effect of the zinc-binding ligand from human milk on zinc absorption as well as of intestinal zinc-binding ligands in infants should provide a better understanding of the primary defect in AE and enhance our basic knowledge of zinc absorption.

CURTIS D. ECKHERT
MARTIN V. SLOAN
JOHN R. DUNCAN, LUCILLE S. HURLEY
Department of Nutrition,
University of California, Davis 95616

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14. We thank Mothers' Milk Bank, Inc., Childrens Hospital, 3700 California Street, San Francisco, Calif., for supplying us with human milk samples, and the Department of Dairy Science, University of California, Davis, for supplying us with unpasteurized cow's milk. Supported in part by funds provided from National Institutes of Health postdoctoral fellowship 1-F32 HD-05219-01 and National Institutes of Health research grant HD-01743.

23 August 1976

Presynaptic Electrical Coupling in *Aplysia*: Effects on Postsynaptic Chemical Transmission

Abstract. *The large cholinergic interneuron L_{10} in the abdominal ganglion of* Aplysia *mediates both chemical and electrical synaptic transmission. The amplitudes of postsynaptic potentials produced by different branches of L_{10} are differentially affected when the electrically coupled neuron L_{20} is depolarized or hyperpolarized. Polarizations applied to L_{20} are transmitted to L_{10} branches by the "presynaptic" electrical synapse. Depolarization increases the amplitude of the postsynaptic potential, while hyperpolarization has the opposite effect. The differential effects occur because current supplied through the electrical synapse undergoes more electrotonic decrement for the distant branches than for branches closer to the electrical synapse. These findings indicate that the presynaptic electrically coupled neuron may have an integrative role in the modulation of chemical synaptic efficacy mediated by L_{10}.*

In the nervous system, transfer of information from one nerve cell to another occurs through the synapse, which may mediate either chemical or electrical transmission (*1*). In the case of chemical synaptic transmission, the action potential arriving at the presynaptic terminal of a nerve cell causes the release of a chemical (transmitter substance) which reacts with a postsynaptic receptor and changes the conductance of the membrane for certain ions; this results in postsynaptic potentials. The conductance changes may cause depolarizing (excitatory) or hyperpolarizing (inhibitory) potentials. The electrical impulse itself is shunted into the low-resistance gap located between the pre- and postsynaptic neurons. In electrically mediating synapses, the gap between the pre- and postsynaptic membranes is very narrow, and hence the resistance between the gap and the extracellular space is relatively high, while the resistance of the electrical pathway between the two neurons is relatively low. An action potential arriving at the terminal crosses the gap and is transmitted electronically with attenuation into the postsynaptic neuron.

Although chemical and electrical synaptic transmission have quite different mechanisms, they have a number of similar functional characteristics (*2*). Presynaptic inhibition, a functional characteristic observed in some chemical synapses, has not been reported in electrical synapses. In this report I show that in the abdominal ganglion of *Aplysia* presynaptic modulation of chemical transmission can be mediated by electrical synaptic transmission.

In the vertebrate nervous system "presynaptic" effects are observed when a test postsynaptic potential (PSP) pro-

duced by activation of one pathway is modified by the activation of another, without concomitant changes in the electrical properties of the postsynaptic neuron. The physiologic observation is generally associated with a particular anatomic serial synaptic arrangement. The terminal of an axon making a synaptic contact with a neuron may itself receive a synaptic input from an axon terminal of another pathway. The latter axo-axonic synapse is then presynaptic to the former. The changes in test PSP are presumably due to the alterations in the amount of transmitter released from the former pathway when the transmitter released from the presynaptic pathway modifies its membrane resistance or spike height, or both (*1*). Similar observations have been made on the crayfish neuromuscular junction (*3*). In the ganglia of *Aplysia*, where all synapses are axo-axonic, synapses occurring near the terminal branches of interneurons could potentially have presynaptic effects. Indeed, in these ganglia there is ubiquitous evidence that most test PSP's produced by stimulation of a nerve (*4*) or activation of a neuron (*5*) may be enhanced or depressed for extended periods of time by stimulation of another nerve. Such effects have been termed heterosynaptic rather than presynaptic, because strict criteria for using the latter term have not been met. Shimahara and Tauc (*6*) have shown that in *Aplysia* heterosynaptic influences are mediated through specific pathways and specific chemical transmitters. So far all presynaptic phenomena observed in both vertebrates and invertebrates have implicated chemical synaptic transmission.

In the abdominal ganglion of *Aplysia* there is a well-known multibranched cholinergic interneuron L_{10}, which has synaptic input on more than 24 other identified neurons in the ganglion (*7*). In addition to its chemical synaptic output, it also has electrical synaptic coupling with at least two other neurons (L_{20} and L_{21}), the characteristics of which have been reported (*8, 9*).

In this study conventional electrophysiological instrumentation and techniques for intracellular recording and stimulation (*7, 8*) were used. Neurons L_{10} and L_{20} (since this cell is more easily accessible than L_{21} and has no input of its own on the other cells studied) and one or two of the follower neurons of L_{10}, such as L_3, L_5, and L_{11}, were impaled with double-barreled electrodes. Hence it was possible to hyperpolarize these neurons with sustained or brief current pulses and observe membrane and synaptic potentials under controlled conditions. The fixed-interval spike activity in L_{10} produced chemical inhibitory postsynaptic potentials (IPSP's) of long latency and uniform amplitudes in L_5 and an electrical excitatory postsynaptic potential (EPSP) of short latency in L_{20}. As illustrated in Fig. 1A, spike activity in L_{10} produces an IPSP in L_5, which is enhanced by about 100 percent when L_{10} activity occurs during a depolarization and spike activity in L_{20} (spikes in L_{20} are not shown). There is a residual depolarization in L_{20} even when the depolarizing pulse is terminated. The IPSP from L_{10} occurring during the residual depolarization remains enhanced by about 10 to 15 percent. It was also noted that the applied pulse in L_{20} had to precede the action potential of L_{10} by at least 0.5 second in order to have any effect on the amplitude of the PSP in the follower neurons.

The relationship between IPSP amplitude in L_5 and hyperpolarization or depolarization in L_{20} is graphically illustrated in Fig. 1B. When L_{20} is at −60 mv (resting potential) the IPSP for L_{10} on L_5

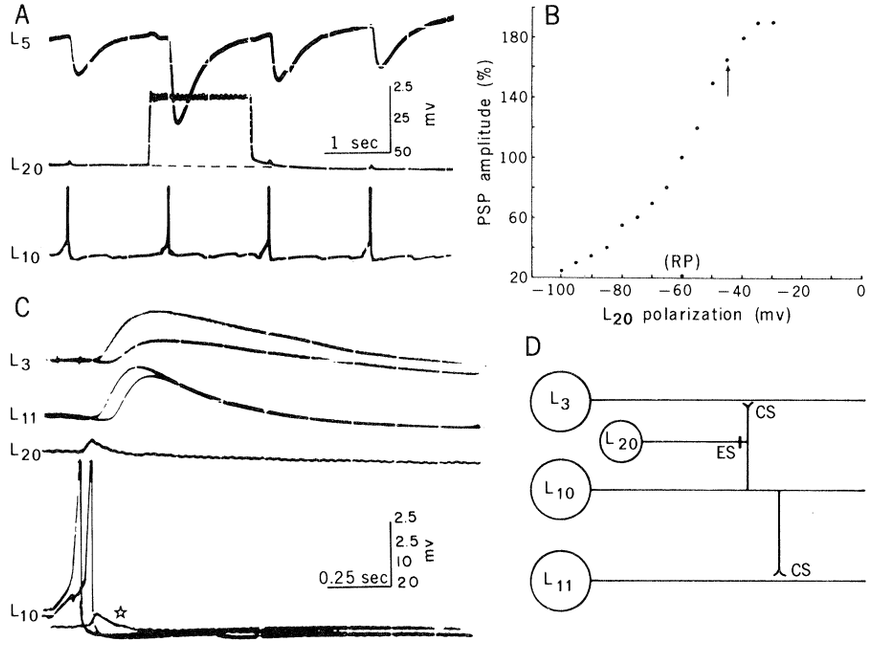

Fig. 1. (A) Effect of a depolarizing pulse applied to the electrically coupled neuron L_{20} on the efficacy of IPSP's produced by interneuron L_{10}. Each action potential in L_{10} produces a chemical IPSP in L_5 and an electrical EPSP in L_{20}. A depolarizing pulse applied to L_{20} causes repetitive spiking (full spikes are not shown) and an increase of about 100 percent in the amplitude of the IPSP in L_5 when the next action potential occurs in L_{10}. Termination of the depolarizing pulse leaves a residual, brief, small depolarization in L_{10}, during which the action potential of L_{10} still produces a slightly enhanced IPSP. Calibration: 2.5, 25, and 50 mv refer to the traces for L_5, L_{20}, and L_{10}, respectively. (B) Effects of applied polarizations in L_{20} on IPSP amplitude from L_{10} on L_5. The resting membrane potential (*RP*) of L_{20} was generally around −60 mv, at which the IPSP amplitude in L_5 was considered to be at 100 percent. Depolarizing pulses of 5, 10, 15, 20, 25, and 30 mv were applied to L_{20} while L_{10} was activated at regular intervals. At −45 mv (arrow), L_{20} began to undergo spike activity. When similar records were obtained with hyperpolarizing pulses, PSP amplitude was gradually decreased. The relation of PSP amplitude to the level of polarization in L_{20} was linear only in the range of polarization where L_{20} membrane potentials were at −45 to −65 mv. (C) Differential effects of L_{20} polarizations on outputs from L_{10}. The IPSP's are recorded from L_3 in the left rostral quadrant and L_{11} in the left caudal quadrant. The first (from left) action potential in L_{10} occurs when L_{20} is at resting membrane potential (−55 mv). It produces IPSP's in L_3 and L_{11} (IPSP's are inverted with sustained hyperpolarization of these two neurons so that their spontaneous activity is blocked and their membrane potential remains unchanged). The second (delayed) action potential of L_{10} occurs when L_{20} has been hyperpolarized to about −85 mv (star) and produces the second pair of PSP's in L_3 and L_{11}, respectively. The amplitude of the second PSP in L_3 has been decreased by about 60 percent, and that in L_{11} by about 20 percent. Calibration: 2.5, 2.5, 10, and 20 mv refer to traces for L_3, L_{11}, L_{20}, and L_{10}, respectively. (D) Schematic representation of the possible spatial relationships between the branches of L_{10} and L_{20} and their synaptic contacts. Neuron L_{10} has a chemical synapse (*CS*) on L_3 and other chemical synapse on L_{11}, and L_{20} has an electrical synapse (*ES*) on a branch of L_{10} near the terminal on L_3. The electrical synapse is presynaptic to the chemical synapses. Polarization in L_{20} will be transferred to branches of L_{10}. Since the terminal in synapsis at L_3 is closer to the source of polarization, it will be affected more than the terminal in synapsis on L_{11}. The latter terminal will be less affected by the transferred polarization because of electrotonic decrement.

is at 100 percent. Stepwise depolarization of L_{20} by 5, 10, 15, 20, 25, and 30 mv enhances the IPSP. Beyond a depolarization of 15 mv, L_{20} begins to undergo spike activity and delayed rectification occurs. With stepwise hyperpolarization of L_{20} there is a progressive decrease in the amplitude of the IPSP from L_{10}. As the curve indicates, the relationship between L_{20} polarization and IPSP amplitude is linear only in membrane potential ranges of −65 to −45 mv. When L_{20} is hyperpolarized to −100 mv, the IPSP is reduced to about 20 percent of the control value. In this experiment further hyperpolarization of L_{10} caused no more decreases in the IPSP amplitudes of this neuron, but the membrane characteristics of L_{20} were adversely affected. However, in other experiments hyperpolarizing L_{20} to −90 mv could totally abolish the IPSP from L_{10}. Also, it was noticeable that in producing hyperpolarizations of more than −70 mv in L_{20} current-voltage relationships were not linear and that L_{20}, like most other neurons of *Aplysia*, manifested anomalous rectification (*7, 10*).

In experiments where I recorded from two follower neurons of L_{10}, such as L_3 and L_{11}, which are in different quadrants of the ganglion (*7*), it was possible to observe the differential effects of L_{20} polarization on the magnitude of changes in the IPSP's produced by L_{10} on these follower neurons. As shown in Fig. 1C, activation of L_{10} produces long-latency chemical IPSP's (inverted) in L_3 and L_{11}. There is also a short-latency (electrical) EPSP in L_{20}. When L_{20} is hyperpolarized by about 25 mv, L_{10} hyperpolarizes by about 2 mv and the activation of L_{10} (second spike) produces IPSP's of lower amplitude in both L_3 and L_{11}. However, the IPSP amplitude in L_3 decreases by about 60 percent, whereas the IPSP in L_{11} is diminished by only about 20 percent. These observations clearly indicated that (i) depolarization or hyperpolarization in L_{20} could modulate the efficacy of synaptic transmission mediated by the multibranched interneuron L_{10} and (ii) that certain synaptic outputs of L_{10}, especially those located in the left rostral quadrant of the ganglion, where L_{20} is also located, could be more effectively modulated than other synaptic outputs, such as those in the left caudal quadrant or in the right caudal quadrant.

The mechanism whereby polarization in L_{20} can modify the amplitude of IPSP's produced by L_{10} is not clear, but certain changes occurring at the terminal branches of L_{10}, as a consequence of L_{20} polarization and anomalously rectifying characteristics of axonal membranes, could ultimately modify transmitter release. In contrast to previous findings in studies of polarization of synaptic terminals, where hyperpolarization increases and depolarization reduces PSP amplitude (*11*), the results of this study show that depolarization of L_{10} terminals increases while hyperpolarization of L_{10} terminals decreases PSP amplitude. Most likely electrotonic spread of polarization from L_{20} to the terminal branches of L_{10} modifies spike height or spike duration, or both, and consequently transmitter release. An important factor that may contribute to the effects of hyperpolarization or depolarization is the occurrence of anomalous rectification (*12*). Hyperpolarization will tend to decrease membrane resistance and possibly the space constant of the terminal branch, reducing the invasion of the synaptic terminal by the action potential, while the opposite will occur with depolarization. Of particular interest is the differential effect of L_{20} polarization on various branches of L_{10}. Since within certain ranges transmitter release by any L_{10} branches is directly related to induced polarization by the electrically coupled neuron, the farther a branch is from the site of electrical coupling, the greater will be the electrotonic decrement and the smaller will be the effects on transmitter release (Fig. 1C). A scheme of such a possible spatial relationship between L_{10} and its follower neurons is presented in Fig. 1D. The electrical synapse is located on the branch or branches supplying input to a group of neurons in the left rostral quadrant represented by neuron L_3. This is likely because L_{20} itself is located in that quadrant. Other branches supplying chemical synaptic output to other neurons such as L_{11}, which are located in the left caudal quadrant and remote from the site of the current-supplying electrical synapse, may be modified less because of electrotonic decrement. The amplitude of polarization reaching the latter synaptic terminals will be less and hence the presynaptic effects of L_{20} will be minimized and, within narrow ranges of L_{20} polarization, may even be absent.

Previous studies have involved direct polarizations of the presynaptic areas, a procedure that may have dubious physiological significance as far as the in vivo integrative functions are concerned. The findings discussed above are a step toward demonstrating that the imposition of such polarizations and the consequent modification of transmitter release may occur by presynaptic electrically coupled neurons intrinsic to the ganglion. At present not enough is known about the input or inputs to L_{20} and their roles in its polarization. In the isolated ganglion, on infrequent occasions, L_{20} is bombarded by large IPSP's and also undergoes waves of depolarizations, which are within the range of imposed polarizations affecting transmitter release by some branches of L_{10}. The role that this multibranched multiaction interneuron plays in the integrative functions of the ganglion has been stressed before (*5, 12*). The findings here point to the existence of electrically mediated synaptic effects on some branches of L_{10} by neurons within the ganglion which may modulate synaptic efficacy in specified pathways supplied by L_{10} and add further complexity and biasing to its variegated functions. In addition, they provide an example of the similarity between electrical and chemical synaptic transmission, as far as presynaptic modulation of synaptic efficacy is concerned.

RAFIG WAZIRI

Department of Psychiatry, University of Iowa Hospitals and Clinics, Iowa City 52242

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13. I thank F. Bahls for reading this manuscript and for his helpful suggestions.

7 July 1976; revised 21 September 1976