observations establish that the high levels of cyclic GMP in the immature *rd* retina are caused by the accumulation of cyclic GMP in the photoreceptor layer.

Our findings indicate that normal photoreceptor cells hydrolyze cyclic GMP by means of their specific high K_m-PDE enzyme and those of the *rd* retina accumulate cyclic GMP due to their deficiency in high K_m-PDE activity. The imbalance in cyclic GMP metabolism of *rd* photoreceptors occurs before the onset of their degeneration and, most probably, causes disturbances in the metabolism or function of these cells. It is premature to speculate on the specific action of cyclic GMP in these cells but, generally, cyclic nucleotides act to regulate cellular function (*8*). This suggests that elevated cyclic GMP will affect the homeostatic balance of the *rd* photoreceptor cells and, if this effect is significant, it could produce changes that result in the degeneration of those cells.

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Presynaptic Inhibition: Primary Afferent Depolarization in Crayfish Neurons

Abstract. *Inhibition of transmission between tactile sensory neurons and interneurons in the crayfish was investigated by intracellular recording in the presynaptic processes. Inhibition is correlated with a depolarization of the presynaptic process, as in the mammalian spinal cord; the depolarization is accompanied by a conductance increase, and is mediated by interneurons that can be excited by a variety of routes.*

Presynaptic inhibition is a process of wide occurrence, in which synaptic efficacy is reduced by preventing the release of transmitter from presynaptic endings, instead of by increasing the conductance of the postsynaptic membrane. In the mammalian spinal cord it is correlated with a depolarization of primary afferent terminals (*1*). The mechanism by which transmitter release is reduced is unknown, however, because intracellular records from the presynaptic axons must be made at a great distance from the terminals, or cannot be made at all (*2*). We here report that primary afferent depolarization (PAD) can be recorded from crustacean sensory neurons at sites close to the region in which they release excitatory transmitter. We have

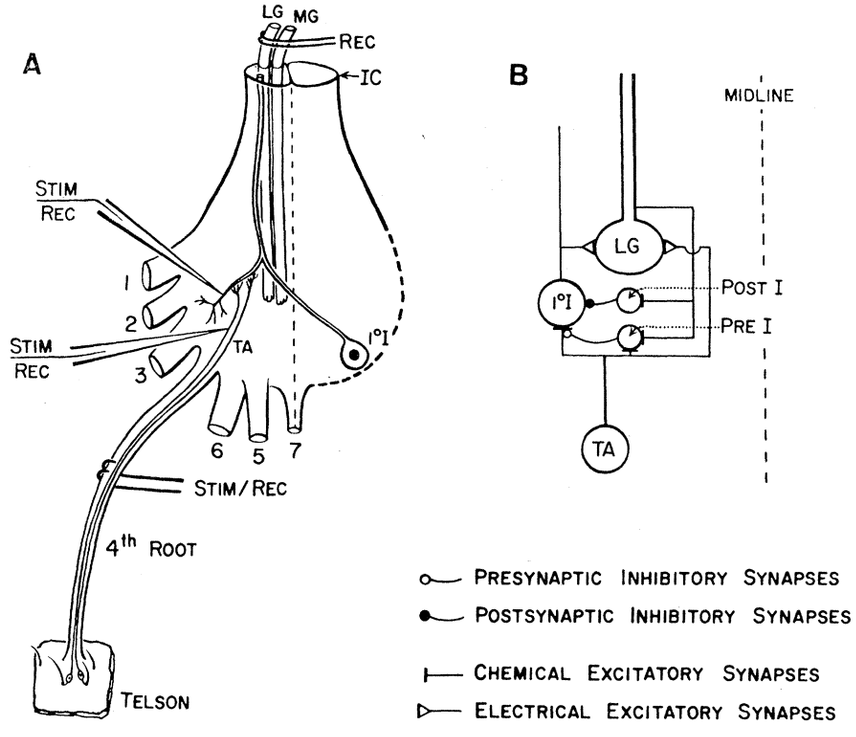

Fig. 1. Preparation and circuit diagram. (A) Composite diagram of the sixth abdominal ganglion preparation (ventral view), displaying the main neural elements and their known anatomical relations. The fourth ganglionic roots, which innervate tactile hairs on parts of the telson and sixth abdominal segment, were left intact. Suction electrodes were used for both stimulation and monitoring of the root activity. The lateral giant fiber was recorded as shown, and stimulated in a more anterior interganglionic connective with a suction electrode. Intracellular electrodes were inserted through the ventral surface of the desheathed ganglion to penetrate either fourth root tactile afferents or first-order tactile interneurons. (B) Simplified circuit diagram indicating known and postulated interactions between the elements discussed in the text. This diagram is based on the work of Zucker (*6*) and Krasne and Bryan (*5*) as well as the experiments discussed here. Connections between the lateral giant and the inhibitory interneurons may be polysynaptic. Abbreviations: *IC*, interganglionic connectives; *LG*, lateral giant fiber; *MG*, medial giant fiber; *POST I*, postsynaptic inhibitory interneuron; *PRE I*, presynaptic inhibitory interneuron; *1°I*, first order tactile interneuron; and *TA*, telson hair tactile afferent. In (A) and (B) only one hemiganglion is shown; all elements are bilaterally symmetrical.

correlated its time course with that of inhibition exerted by known pathways against first-order synapses, and measured an associated conductance change in the afferent terminals.

Earlier observations have suggested that a presynaptic inhibitory process operates against sensory endings in the neuropil of crustacean ganglia. Excitatory postsynaptic potentials (EPSP's) evoked in interneurons show long-lasting inhibitory interactions (*3*), and depolarizations have been reported from afferent fibers in the lobster brain, although not in association with functional inhibition (*4*). Krasne and Bryan (*5*) showed that impulses in the central giant fibers of crayfish produced a "remote" inhibitory effect on transmission from tactile afferents to interneurons, as well as inhibitory postsynaptic potentials. They proposed that the remote inhibition was presynaptic, and suggested that it protects the synapses, known to be vulnerable to habituation (*6*), from loss of efficacy during bouts of sensory discharge evoked by rapid escape movements.

We isolated the ventral nerve cord of crayfish (*Procambarus clarkii*) except for the fourth roots of the sixth ganglion, which remained attached to the tail appendages they innervate (Fig. 1A). In this preparation, the exoskeletal hairs that supply afferent axons to the sensory nerves are available for mechanical stimulation. The cord was pinned out ventral side up in a chamber lined with transparent Sylgard resin, desheathed, and illuminated from below. Suction electrodes on the sensory roots were used to monitor activity evoked by stimulating sensory hairs or to supply brief electrical shocks to the afferent root, and suction electrodes on more anterior connectives were used to monitor interneuronal activity or to excite large, identified command interneurons (the lateral giant fibers). A simplified circuit diagram identifying the main elements to be discussed and indicating their mode of interaction is shown in Fig. 1B.

Glass capillary microelectrodes (40 to 70 megohm), pulled with fiber glass wisps and filled with 3*M* KCl, were used to impale units in the neuropil of the sixth ganglion. The following criteria were applied to identify a recorded unit as an afferent process: (i) direct impulses, without prepotentials, could be evoked by electrical stimulation of the fourth or fifth roots, or injected depolarizing currents produced impulses propagated out one of the fourth roots (or both); and (ii) impulses could be evoked directly in the impaled unit by manipulating single sensory hairs. Although all units reported here met the first criterion, some could not be discharged by natural stimuli because part of the fourth root's innervation field is unavoidably excised in making the preparation (*7*). All observations reported on such cells, however, have been verified on afferents with identified peripheral terminations.

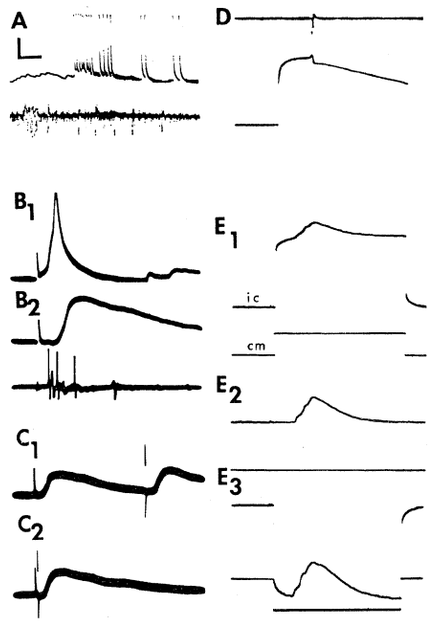

Fig. 2. Electrical responses recorded intracellularly from afferent axons in sixth ganglion neuropil. (A) Upper trace, depolarizations and spikes in an afferent axon produced by tactile stimulation of its receptive field; lower trace, extracellular record from the sensory root (calibration: 10 mv, 100 msec). (B) Comparison of the latency of EPSP's in an interneuron (B_1) with that of PAD in a neighboring afferent process (B_2), evoked at the same intensity of electrical stimulation applied to the fourth root. The lower trace in B_2 is a record from the connective between the fifth and sixth ganglia showing ascending discharge in interneurons activated in the sixth ganglion (5 mv, 5 msec). (C) Occlusion between PAD produced by shocks to the connective (first stimulus in C_1) and the fourth root (second stimulus). The two shocks are made to coincide in C_2 (10 mv, 10 msec). (D) Production of a propagated spike in the fourth root (top trace) by depolarizing pulse injected through the recording microelectrode (bottom trace) (50 mv, 10 msec). (E) Changes in amplitude of PAD produced by altering membrane potential. The PAD was produced by giant fiber stimulation; the membrane was polarized by injecting depolarizing (E_1) and hyperpolarizing (E_3) currents. Top traces, intracellular (*ic*) records from an afferent process; bottom traces, current monitor (*cm*) (10 mv, 1 na, 10 msec). (A), (B), and (C) are from the same neuron, (D) and (E) are from a different one.

The inhibitory phenomenon that we presume to be presynaptic can be recorded from many interneurons that are activated monosynaptically by afferents from the fourth root (*8*). A brief train of impulses (at 100 hertz) in the lateral giant fiber will reduce the amplitude of a test EPSP in some interneurons by 75 percent; yet in some of these cells no postsynaptic potential change is produced by the train, even when the membrane potential is depolarized by up to 20 mv by current injected using a bridge circuit.

When penetrations are made in the presynaptic primary afferent neurons, graded shocks applied to the sensory nerve or natural stimuli produce small depolarizations below the threshold for a directly evoked impulse in the impaled neuron (Fig. 2, A and B). Above the threshold for a direct impulse, the PAD shows further augmentation with increases in the intensity of the stimulus to the afferent root. Similar subthreshold responses are obtained by stimulating the central connectives, especially the lateral giant fibers (Fig. 2, C and E).

The PAD evoked by sensory nerve stimulation has a latency of approximately 3 msec from the calculated arrival time of the volley in the ganglionic neuropil. This value is at least 2 msec longer than the latency of monosynaptic EPSP's produced by the same input recorded in interneurons penetrated near the same afferent processes (Fig. 2B). The latency of PAD is long enough to permit the discharge of interneurons before its onset (Fig. $2B_2$). Furthermore, unitary depolarizing potentials in the afferent terminals are not associated with single impulses simultaneously recorded from axons in the sensory nerve generating the PAD, although such impulses clearly generate unitary EPSP's in interneurons with which the sensory axons connect monosynaptically (*8, 9*). Primary afferent depolarizations nevertheless appear to consist of elementary components (Fig. 2, A and C); these are recruited in discrete fashion by gradations in stimulus intensity. When maximal PAD's produced by stimulation of an afferent root and the cord pathway are timed so as to occur simultaneously, occlusion occurs (Fig. 2C). All these findings indicate that afferent fibers do not

end directly on one another, but that instead a limited number of intervening interneurons mediate the depolarizing responses.

We analyzed the conductance change associated with PAD by recording PAD amplitudes during the injection of steady hyperpolarizing and depolarizing currents. The former increase, and the latter decrease, PAD amplitude (Fig. 2E). Neither the bridge balance nor the position of the microelectrode with respect to the synaptic region can be exactly known, but the relation between membrane potential and PAD amplitude crudely extrapolates to a reversal potential 18 mv below resting potential; other experiments gave values between 12 and 20 mv. This suggests that a change in Na^+ conductance plays a part in PAD, and we have observed that PAD can sum with injected depolarization to generate impulses.

To compare the time course of PAD with that of the inhibition exerted against the transmission link between primary afferent neurons and interneurons, we successively penetrated a sensory fiber showing PAD and then, nearby on the same microelectrode track, an interneuron activated monosynaptically by the afferent fiber group to which the sensory unit belonged. We compared the latency and time course of the inhibitory effect with that of PAD, using the central giant fibers to generate each. The results are plotted in Fig. 3. The peaks of PAD and of the inhibitory effect coincide in time, but the latter effect somewhat outlasts the former (*10*).

Is it the depolarization itself, the conductance change associated with it, or neither that is responsible for the inhibition? In the mammalian spinal cord, PAD is recorded several space constants away from its source, where it cannot be affected by injected current and where any conductance changes are too remote to be detected. We have evoked compound PAD's by brief trains of stimuli to the lateral giant fibers, and find that under favorable conditions—where the PAD is large—considerable shunting of the afferent spike occurs (Fig. 4). This result suggests that we record close to the sites at which the afferents release their transmitter and indicates that shunting of the spike is a candidate mechanism for the presynaptic inhibition.

Presynaptic depolarization thus appears to be associated with inhibition in the same way as it is in the mammalian spinal cord; this finding provides an explanation of the observation (*5*) that central motor pathways prevent the loss of synaptic efficacy during repetitive movements. A restricted pool of interneurons, sharing a number of inputs, appears to control central excitability at a primary level; these may be responsible for a number of the hitherto unexplained excitability fluctuations reported in interneurons of unrestrained animals (*11*). The large sensory fibers we have studied—in contrast to afferent axons in the mammalian dorsal horn—can be penetrated near their sites of transmitter release, so that conductance changes associated with presynaptic inhibition are measurable. They invite experiments on the ionic mechanism of this process that have not been possible in mammalian systems.

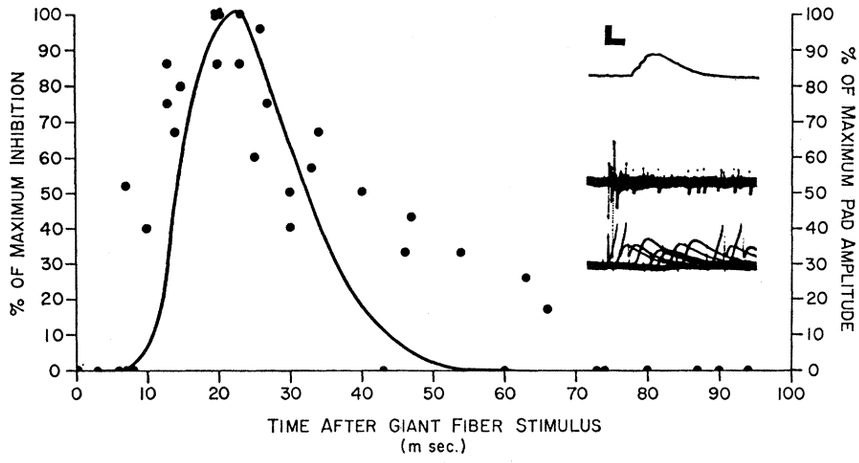

Fig. 3. Comparison of the time courses of PAD and the inhibition exerted by the same pathway against synaptic transmission from afferent fibers to interneurons. (Inset) Upper records show PAD recorded in an afferent process in response to giant fiber activity; lower records show EPSP's and spikes generated in an interneuron by shocking the fourth root. At the start of the lower record, the giant fibers were shocked; this was followed by the test stimulus to the root, and a series of intervals are shown in the superimposed traces. Below about 15 msec, the EPSP's produce spikes which incompletely repolarize the synaptic potentials. At 25 msec, EPSP amplitude is minimum, and it increases again and ultimately generates spikes at the two longest intervals. The upper trace is a monitor on the 5-6 connective (calibration: 10 mv, 10 msec). In the graph, the solid line is the averaged wave form of five PAD records; the points are the measured amplitudes of EPSP's. Both were measured from series of records like the ones in the inset and normalized. The inhibitory pathway produces post- as well as presynaptic effects in this cell (*4*).

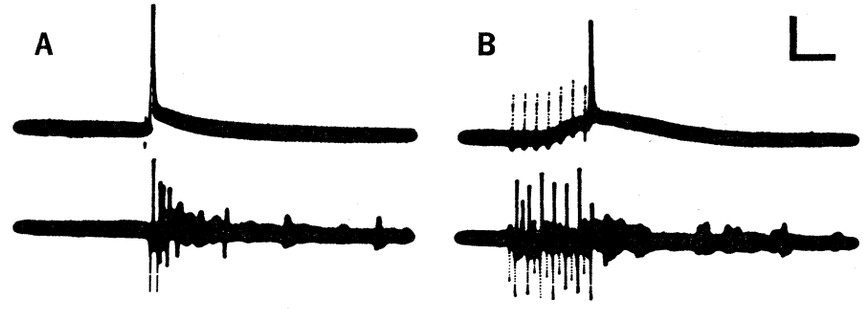

Fig. 4. Shunting of the amplitude of a direct impulse in an afferent fiber by the PAD produced by repetitive stimuli to the lateral giant axon. In (A) a shock to the fourth root produces an impulse in the impaled cell; in (B) the same stimulus is inserted at the peak of the compound PAD produced by a train of six lateral giant impulses monitored in the 5-6 connective (bottom traces). Calibration: 20 mv (intracellular records), 10 msec.

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7. The fourth root of the sixth ganglion does contain a single efferent neuron. It is subject to synaptic activation by a variety of segmental inputs and, like other motoneurons, responds in a way entirely different from the penetrations described here. The fourth root afferents that are disconnected in these experiments are among the largest sensory fibers entering the sixth ganglion, which probably explains why they were commonly encountered.
8. Connections are judged to be monosynaptic on the basis of latency (< 0.5 msec) and the presence of unitary EPSP's associated with single afferent impulses [see R. S. Zucker, D. Kennedy, A. I. Selverston, *Science* **173**, 645 (1971)].
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10. The discrepancy between the duration of PAD and of the inhibitory effect has at least two possible explanations. First, interneurons like the one tested probably receive a mixture of pre- and postsynaptic inhibition (*5*), and the latter may involve long polysynaptic pathways. Second and more likely, although the presynaptic conductance change measured in our experiments is a possible mechanism for presynaptic inhibition, it is not necessarily the only one. If, for example, inhibition of transmitter release involved changes in the amount of C^{2+} available at the terminal, the effect could outlast measurable changes in membrane potential or conductance.
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Evidence for in vivo Reaction of Antibody and Complement to Surface Antigens of Human Cancer Cells

Abstract. *The immune adherence test was used to determine whether antibody and complement in cancer patients are fixed in vivo to tumor cells. Human erythrocytes adhered in vitro to the surface of human cancer cells obtained from autopsy and biopsy. Adherence was enhanced by further addition of the C2 and C3 components of complement, and was diminished by preliminary treatment with antibody to C3 (that is, to β1C-globulin). The results suggest that tumor associated membrane antigens form complexes in vivo with antibodies and complement.*

Recent studies have shown that malignant human tumors possess tumor specific surface antigens and that patients with tumors do produce humoral antibodies against these antigens (*1*). Various immunologic techniques indicated that humoral antibodies from the serums of cancer patients react with antigens on the membranes of cancer cells in vitro. However, it has not been established whether such reactions take place in vivo. In fact, it has been suggested that antibodies cannot cross the capillary barrier in sufficient amounts to influence tumor growth patterns.

We assumed that, if tumor associated antigens formed complexes with antibody and complement on the membranes of cancer cells in vivo, such complexes would be detectable by the immune adherence (IA) phenomenon in vitro. Ever since Nelson's discovery that normal human erythrocytes adhered to complexes of antigen, antibody, and complement on a cell membrane (*2*), this sensitive immunoassay has been used to detect and characterize membrane antigens in animal systems (*3*) and, more recently, in human systems (*4*). We have tested the in vitro reactivity of various human cancer cells obtained from biopsy and autopsy.

Biopsy specimens from 12 patients with various malignancies and normal tissues from 8 patients who died of cerebrovascular or cardiac diseases were used. Free cells were prepared as described (*1*). The cells were frozen at 1°C per minute in a fluid containing RPMI medium 1640, 10 percent dimethyl sulfoxide, 20 to 40 percent human serum from which the γ-globulin has been removed (human agamma serum; Irvine Scientific Sales), penicillin, streptomycin, and Fungizone (Grand Island Biological) and stored at − 190°C (liquid nitrogen freezer). The cells were thawed rapidly at 37°C in a shaker bath, washed once with a medium containing RPMI 1640 and human agamma serum (20 to 40 percent) and three times with barbital-buffered saline containing Ca^{2+}, Mg^{2+}, K^+, glucose, and human albumin (buffered saline 1) (*4*), and resuspended in this buffered saline for use as target cells. The contaminating erythrocytes were eliminated by freezing and thawing and subsequent low-speed centrifugation.

Human erythrocytes carrying IA receptors from a healthy O-type donor were used as indicator cells (*4*). Sheep erythrocytes were prepared by the same procedure and used as a control for the indicator cells.

A portion (50 μl) of the human erythrocyte suspension (4×10^6 cells) was added directly to 25 μl of the target cell suspension (2.5×10^4 cells) without any serum or complement. This mixture was incubated at 37°C for 10 minutes with agitation, and for 20 minutes without agitation. One drop of this mixture was placed on a glass slide, covered by a cover slip, and viewed through the microscope. The IA patterns were determined by the percentage of target cells that positively adhered to human erythrocytes in a count of 50 cells. Adherence of at least one erythrocyte to the target cell was taken as an IA-positive reaction. To distinguish a true positive from a pseudopositive pattern, the cover slip covering the reaction mixture was lightly tapped with a pencil. The erythrocytes adjacent, by chance, to target cells moved away, while the truly positive ones remained attached.

In order to prove that the attachment of erythrocytes to cancer cells was indeed the IA phenomenon, three experiments were performed. In the first, the reactivity of the cancer cells was enhanced by adding purified human complement 2 (C2) and complement 3 (C3); in the second, the reactivity was inhibited by prior treatment with antibody to human β1C-globulin (that is, antibody to human C3) (*5*); and in the third, the specific adherence activity was tested against sheep erythrocytes to the target cancer cells (*2*).

The first experiment was necessary because in the IA phenomenon a human erythrocyte adheres to a C3 site in a complex of antigen, antibody, and complement components C1, C4, C2, and C3 (fixed in that order) (*2*). The C5 and subsequent components are not essential. Since it is known that C2 decays rapidly from the complex at 37°C unless stabilized by C3 (*6*), additional complement was added to overcome the possible loss of C2 during preparation of the cell suspensions, and