Inhibition of Mechanosensory Interneurons in the Crayfish. I. Presynaptic Inhibition From Giant Fibers

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SUMMARY AND CONCLUSIONS

1. Sucrose-gap and intracellular recordings were used to study the primary afferent depolarization (PAD) produced in mechanosensory afferents by impulses in lateral and medial giant axons, which are the command cells for the tail flip escape response in the crayfish.

2. The lateral and medial giant axons produce PAD through a polysynaptic interneuronal pathway. The response has a relatively long intraganglionic latency (7-11 ms), and command-evoked PAD can be recorded in ganglia from which the giant axons have been experimentally disconnected.

3. The final neurons of the pathway that delivers inhibition are few in number and extensive in distribution; most appear to be common to lateral and medial giant pathways.

4. At least some of the inhibitory interneurons have axons in the interganglionic connectives and probably produce both presynaptic and postsynaptic inhibition.

5. Stimulation of the lateral, but not the medial, giant axons causes a small, short-latency depolarization that is stable at high repetition rates. This small potential can be accounted for by transmission across known electrical synapses between mechanosensory afferents and the lateral giants in each abdominal ganglion.

6. Repetitive stimulation of the lateral giant axons causes substantial augmentation of PAD, apparently through recruitment of additional interneurons. PAD evoked by a single medial giant (MG) stimulus is generally much larger than that elicited by a single lateral giant (LG) spike. However, MG-PAD summates little and so the maximum PAD ΔV reached during repetitive firing is equivalent for the two types of giant axons.

7. Iontophoresis of γ-aminobutyric acid (GABA) into the ganglionic neuropil depolarizes the primary afferents and blocks activity in neurons that have axons in the interganglionic connective.

8. The extrapolated PAD reversal potential and pharmacological studies suggest that a GABA-mediated chloride conductance increase is involved in the production of PAD.

INTRODUCTION

Rapid movements are often accompanied by feedback or feedforward inhibition of associated sensory structures. This principle operates, for example, in the phenomenon of saccadic suppression (1), in the efferent inhibition of auditory signals associated with vocalization (29), and in the suppression of mechanosensory activity that accompanies rapid escape movements in lower vertebrates (7, 25) and invertebrates (4, 5, 16, 17).

In one instance, that of rapid escape movements in the crayfish, both the source and target of the inhibition have been clearly identified. Krasne and Bryan (17) first showed that the giant command neurons for escape could initiate a powerful
descending inhibitory action on the first-stage synapses between receptors for near-field water disturbances (35) and identified interneurons. They also demonstrated that the inhibition protected the synapses from the depressive effects of repetitive activation and concluded that inhibition must include a presynaptic component. Subsequently, we made intracellular recordings from the afferent neurons near their zones of termination in the sixth abdominal ganglion (16), and showed that primary afferent depolarization (PAD) was associated with the inhibitory effect. Although the time course of PAD was shorter than the period during which excitatory postsynaptic potentials (EPSPs) in neighboring primary interneurons were reduced, the conductance change associated with the depolarization shunted the peak amplitude of afferent impulses by 3–5 mV. We hypothesized that PAD is the electrophysiological correlate of presynaptic inhibition.

Our preliminary report of the presynaptic inhibitory circuit left many questions unanswered. It may be wondered, for example, what kinds of ionic permeability changes underlie the conductance increases. It is, furthermore, not at all clear what kind of interneuronal circuit actually delivers the inhibition to the afferent fibers. The giant command neurons are clearly presynaptic to other interneurons (18, 36); are these interneurons directly inhibitory, and do the same ones deliver inhibition postsynaptically and presynaptically? Finally, what are some of the integrative properties of this interneuronal circuit?

In this paper we report the results of experiments aimed at these questions. We have used the technique of sucrose-gap recording from afferents in order to record PAD as a population response from many sensory cells. The results show that a relatively small number of inhibitory interneurons deliver presynaptic inhibition to all of the target afferent endings, and it is likely that in some cases the same inhibitory interneurons produce inhibition that is both presynaptic and postsynaptic with respect to interneurons in the near-field mechnosensory system. GABA is the putative transmitter for PAD, and increased chloride conductance appears to be responsible for the potential change.

**Method**

Crayfish (*Procambarus clarkii*, both sexes) 7–10 cm long were used in making both isolated nerve cord preparations and minimally dissected preparations. In one set of experiments the abdominal nerve cord was isolated and pinned out in a sylgard-lined preparation dish (Fig. 1) that consisted of two chambers connected by a thin channel. A small strand of silk thread was tied to the end of the fourth root of the sixth ganglion and used to pull the root through the groove in the wall of the first chamber, over the channel, and into the second chamber. The grooves in both chambers were sealed with Vaseline, the chambers filled with crayfish saline (33), buffered to a pH of 7.2 using Trizma, and the channel was filled with an isosmotic (450 mM) sucrose solution. The two chambers were, thus, isolated electrically except for the nerve; they were recorded from differentially, using WPI electrode holders filled with saline. The differential input was amplified by a Tektronix type 122 preamplifier using a coupling time constant of 1 s (where absolute voltage levels were unimportant) or by a DC preamplifier. Glass suction electrodes with varying tip diameters were used to stimulate selected fibers and monitor the nerve cord activity. Typically, giant axons were stimulated in the desheathed connective between the second and third ganglia using a small, focally placed electrode; impulse activity was monitored en passant in the desheathed connective between ganglia five and six to verify that the identified giant fiber was selectively activated. Under these conditions of stimulation, the giant fibers have much lower thresholds than other units in the connective. Recordings from single interneurons were made by gently dissecting the fibers from the five–six connective with fine forceps and then drawing up the rostral end into a fine-tipped suction electrode. For experiments in which intracellular recording was necessary, the sixth ganglion was desheathed and penetrated ventrally with glass microelectrodes filled with 3 M KAc or KCl. Electrode resistance was between 30 and 70 MΩ. A bridge circuit was used for current injection.

For transmitter studies, microelectrodes were filled with 4.0 M GABA at pH 4.5. The micropipettes were inserted into an Ag/AgCl bridge (WPI electrode holder) and connected to a stimulator that could deliver pulses of any duration over a continuously variable range of
FIG. 1. Overview of the experimental preparation. A: arrangement for stimulation of giant axons, simultaneous extracellular recording of interneurons and afferents, and sucrose gap recording of afferents in the fourth root of the sixth abdominal ganglion. All intracellular recordings reported in this paper were made from mechanosensory afferents and interneurons in the sixth abdominal ganglion. B: schematic diagram of the circuitry investigated. It is not intended that every CDI, II, or I° int be thought of as receiving all connections shown. LG, lateral giant; MG, medial giant; CDI, corollary discharge interneurons; II, inhibitory interneurons; I° aff, primary mechanosensory afferents; I° int, primary mechanosensory interneurons.

RESULTS

Additional evidence for presynaptic inhibition

So far, as indicated in the INTRODUCTION, the evidence for presynaptic inhibition of afferents has been indirect; the analysis
has been complicated because the only first-order interneurons previously studied also received postsynaptic inhibition. Additional evidence for presynaptic inhibition is shown in Fig. 2. These records are from an unidentified first-order mechanosensory interneuron penetrated in the neuropil of the sixth ganglion. The lateral giant (LG) axons, which are the "command neurons" for one kind of crayfish tail flip response, were stimulated to fire a train of impulses while the interneuron was depolarized with injected current. The LG train failed to produce any observable potential change in the interneuron (Fig. 2A).

In spite of the absence of any observable postsynaptic inhibition, the train of LG impulses reduced the amplitude of EPSPs evoked by fourth-root stimulation to about one third of their control value (Fig. 2B). It is possible that this neuron receives postsynaptic inhibition at a point too remote from the recording site to be detected, but close enough to the EPSP site to influence the latter. This alternative seems unlikely in view of the lack of any change in the time course of the EPSP.

Sucrose-gap recording of primary afferent depolarization

The fourth root of the sixth abdominal ganglion consists entirely of sensory axons, with the exception of a single small, unidentified efferent (6). Recordings made with a sucrose gap across the fourth root, a few millimeters from the ganglion, should, therefore, primarily record potential changes in the afferents within the ganglion.

Records shown in Figs. 3 and 4 illustrate directly that the population responses recorded in this way correspond to those recorded intracellularly from representative afferent neurons in the same root. The upper traces are from the sucrose gap; the lower traces are from a microelectrode that has penetrated a fourth-root afferent within the sixth-ganglion neuropil. In Fig. 3A1, injected depolarizing current was delivered to discharge the afferent, and the blocked spike was recorded by the sucrose-gap electrode. When a directly elicited medial giant (MG) impulse preceded the current pulse by an appropriate interval, it produced PAD (seen in the sucrose-gap record)
FIG. 3. PAD is associated with a conductance increase of the afferent terminals. Simultaneous sucrose gap (upper traces) and intracellular records (bottom traces). In A1, the penetrated afferent was depolarized to its threshold for impulse initiation by a 40-ms depolarizing current pulse injected through the recording microelectrode. In A2, conditions were identical except that a stimulus to the medial giant axon evoked primary afferent depolarization (PAD) (top trace) and inhibited spike generation. The experiment was repeated in B with a larger depolarization and stimulation of the lateral giant. Note that PAD is reversed by the polarization, but because of electrode rectification the bridge was not balanced, so that the voltage measure is not accurate. In these and many of the following records, PAD appears biphasic because recordings were AC coupled.

and eliminated the discharge in the afferent, indicating that the MG circuit had increased membrane conductance in the afferent (Fig. 3A). In Fig. 3B the experiment was repeated using a larger depolarizing current that induced repetitive activity, and PAD was produced by evoking a lateral giant (LG) impulse. The pause in the evoked spike train corresponded in duration to the PAD recorded across the sucrose gap. The PAD recorded via sucrose gap exhibited the same latency and time course as the intracellularly recorded response to giant-axon impulses, which at the imposed level of membrane potential was hyperpolarizing.

In Fig. 4, consecutive sweeps of a simultaneous intracellular record (lower traces) and a sucrose-gap record are shown. The responses were to single impulses in the MG axon but, as often happens, there was step variation in the amplitude of the responses. When the additional component was present in the intracellular record, it was also present in the sucrose-gap record. Good agreement between the component structure of sucrose gap and intracellularly recorded PAD was found consistently in a long series of responses. In general, LG-evoked or MG-evoked PAD consists of a minimum of two or three and a maximum of about six unitary components, whether one is recording intracellularly or extracellularly.

**Differences between PAD evoked by lateral and medial giant impulses**

Impulses in either lateral or medial giant axons characteristically evoke multicomponent PAD in the fourth-root afferents. The responses, however, show consistent differences (Fig. 5). Selective stimulation of the MG axon with a fine suction electrode (Fig. 5A) evokes PAD of large amplitude with a latency of about 9 ms. Stimulation of the LG axon (Fig. 5B) causes a smaller, short-latency (2 ms) component, followed by a larger component. The onset of the latter is obscured somewhat by the first component, but its latency is several milliseconds longer than that of the PAD evoked by MG stimulation. The early component reliably distinguishes the LG response in all preparations we have examined (cf. Fig. 3A, and B).

The early component faithfully follows repetitive stimulation at the LG axons at frequencies of 200 Hz or higher without changes in amplitude or latency. It therefore represents coupling across the electrical synapses known to link these afferents and the lateral giant (40). In contrast, the MG axons are not known to receive any synaptic input within the abdomen. The late response of PAD decreases conspicuously

FIG. 4. Comparison of sucrose-gap recording from a population of afferents (top trace) and a simultaneous intracellular recording of a single tactile sensory neuron (bottom trace). Units of PAD are evoked by stimulation of the medial giant (MG) axon. In B, an extra component of PAD appears in both records (arrow).
FIG. 5. Comparison of PAD produced by MG (A) and LG (B) impulses. Upper traces are extracellular records of the giant axon impulses recorded in the five-six connective, lower traces show PAD recorded across the sucrose gap. The small depolarizing potentials in the sucrose-gap record are impulses in the single efferent neuron in the fourth root. The giant axons were stimulated in the two-three connective.

at such frequencies and also shows trial-to-trial variation in the number of unitary components recruited at much lower stimulation frequencies. In both of these respects the late LG response resembles the MG response, and occlusion occurs between the two (see below).

Other interganglionic interneurons mediating PAD

Single impulses in the LG or MG axons recruit discharges in many other interganglionic interneurons (36). Several of these interneurons have been identified (18) and been shown to evoke components of PAD. However, the evoked PAD occurs at long latency (18), indicating that these elements are probably triggering the same polysynaptic, PAD-producing circuitry that the giant axons activate. We attempted to locate interganglionic interneurons that might be more directly linked to the final inhibitory interneurons, as indicated by short latencies and stable coupling.

In our experiments the giant axons were interrupted in the five-six connectives and the rostral portions of the axons were stimulated. In addition to PAD, the activity of surviving interganglionic interneurons was recorded between ganglia five and six; these represented the only component of the intraganglionic PAD circuitry still intact. Although many interneurons could be found in this way, their association with PAD could usually be shown to be polysynaptic because stimulating the giants at high frequency caused PAD components to fail, while the interneuron impulse remained.

However, on three occasions we found small interneurons that were tightly linked at short latency with components of PAD. Figure 6 is an example of such an experiment; in this instance, all four giant axons had been stripped from the five-six connectives. The latter were then searched with a small suction electrode while the two-three connective was stimulated, until a descending impulse was found that reliably preceded the PAD component. The cord was then stimulated at high intensity several segments away to excite the unit directly. Its conduction velocity was not determined precisely, but the long latency of the descending spike indicates that it was quite slow. The presence of the PAD component and the impulse were correlated one to one: at high stimulus frequencies, occasional

FIG. 6. An interganglionic interneuron that produces PAD at short latency. Bottom traces are records of interneuronal activity in the desheathed five-six connective after stripping out the giant axons. Upper traces are sucrose-gap recordings of the fourth root. A: shock to the ventral surface of the three-four connective just below threshold for the interneuron. B: a stimulus just above threshold for the interneuron (marked by a triangle) followed at short latency by PAD.
failures of the descending impulse were invariably accompanied by failure of a PAD component. The latency between the impulse recorded by the monitoring electrode and the PAD was about 1.7 ms, some of which must be occupied by the considerable conduction time between the monitoring site and the ganglionic neuropil. These findings are consistent with a monosynaptic inhibitory connection between some interganglionic interneurons and sixth ganglion afferents.

Properties of interneuronal network

The multicomponent nature of primary afferent depolarization, seen both in individual afferents and in the population response, suggests that PAD is directly mediated by a small number of interneurons, each of which delivers presynaptic inhibition to most or all of the fourth-root mechanosensory afferents. It now appears that a variety of pathways converge on the interneurons that produce PAD. Earlier experiments (16) demonstrated occlusion between sensory-evoked and giant fiber- (GF) evoked PAD in single afferents. There also seems to be substantial occlusion between MG-PAD and LG-PAD, since the maximum PAD amplitude when both fibers are stimulated is no larger than the PAD produced in response to shortest interval pairs of shocks to either giant alone (Fig. 8). It is unlikely that the apparent occlusion simply reflects a saturated PAD amplitude at the equilibrium potential of the PAD conductance because the sum of the PADs produced by each giant alone is less (by about 10–15%) than the PAD recorded when both giants are stimulated at the same time (Fig. 8). In a subsequent paper it will be shown that occlusion clearly occurs between giant axon-evoked PAD and PAD produced by an entirely separate pathway. These results indicate that there is a final common path for presynaptic inhibition involving a relatively small number of elements with widespread output connections to mechanosensory afferents.

Substantial integrative action takes place within this pool of inhibitory interneurons. In occasional preparations, single giant-axon impulses produced no detectable PAD, whereas the combined stimulation of MG and LG axons often did. Even when single giant-axon impulses produced PAD, repetitive stimulation gave rise to dramatic augmentation. In Fig. 7, single LG impulses produced a small PAD with an early component (Fig. 7A), while a train of four impulses at 200 Hz produced a huge increase in PAD magnitude.

A graphical representation of similar augmentation in another experiment is shown in Fig. 8. Notice that the PAD produced by single MG stimuli (control) is considerably larger and more variable than that produced by single LG stimuli. It is also evident that the amount of amplitude augmentation produced by repetitive stimulation is inversely related to the PAD amplitude produced by single giant-fiber impulses.

PAD evoked by single MB impulses was occasionally small; like that produced by LG impulses (e.g., Fig. 5). In the two instances in which this occurred, MG-PAD amplitude showed augmentation equal to that seen for the LG-PAD. On the other hand LG-PAD was never observed to be large and to show little augmentation, as we often found to be the case when PAD

![Fig. 7](image-url) Augmentation of PAD by multiple giant-axon impulses. PAD recorded via sucrose gap from the fourth root, in response to single LG impulses (A) and brief trains (B). Bottom traces are records of the five-six connective, showing stimulus artifacts (arrows) and LG impulses (diamonds).
was produced by stimulating the MG. The possible significance of the difference in the way the two giant fibers recruit elements of the PAD-generating network will be discussed in a later paper in this series. For now, the point to be emphasized is that there may either be different pools of preinhibitory interneurons for the two giant axons or the giant axons might, for a variety of reasons, have different efficacies for activating the same preinhibitory interneurons. A few preinhibitory interneurons that have been identified so far appear to be driven by both giants (18). If the final inhibitory interneurons activated by the two giant fibers are identical, as our occlusion data suggests, all of the integrative differences between the two giant fibers with regard to the network must occur at the level of the preinhibitory interneurons.

Presynaptic and postsynaptic effects attributable to same interneurons

We tried to evaluate whether inhibitory postsynaptic potentials (IPSPs) in interneurons could be associated with components of PAD in related afferents. In these experiments we stimulated a ventral inhibitory pathway that converges on the same inhibitory interneurons as the giants (unpublished observations). At the same time the sixth-ganglion neuropil was probed with microelectrodes for mechanosensory interneurons that received descending inhibition. PAD was simultaneously recorded from fourth-root afferents. In three such experiments, the component structure of the PAD recorded across the sucrose gap was correlated with the appearance of the IPSPs recorded from the interneuron (Fig. 9). The upper traces are sucrose-gap recordings of PAD; the lower traces are intracellular records from an unidentified sensory inter-
neuron. In each record the stimulus artifacts mark a shock to a ventral, inhibitory pathway (unpublished observations); the giant axons were not activated. In Fig. 9, A and B each represent four consecutive superimposed sweeps at a stimulus rate of 50 Hz; at the beginning of each sweep, a long pulse of depolarizing current was injected into the interneuron through the recording microelectrode in order to increase the amplitude of the hyperpolarizing IPSPs. The latencies of IPSPs and PAD components are identical and stepwise variations in their amplitude are perfectly correlated with one another. Although the difficulty of doing these experiments precluded our exploring these relationships more rigorously (it would have been desirable, for example, to work only with the shortest latency, most direct interneuronal pathways and to try various maneuvers for dissociating the IPSP and PAD components), the results are consistent with the idea that at least some of the interneurons in this network deliver both presynaptic and postsynaptic inhibition.

Effect of GABA and its antagonists, picrotoxin and bicuculline

Since \( \gamma \)-aminobutyric acid (GABA) is the transmitter for both presynaptic and postsynaptic forms of peripheral inhibition in Crustacea (8–10, 23, 30, 31, 32) and is a putative transmitter for postsynaptic inhibition centrally, we tested the effect of GABA and its antagonist, bicuculline, on PAD. Bicuculline \((10^{-5} \text{ M})\) was found to reduce PAD by 80% within 10 min following its addition to the saline bathing the preparation. Maximum recovery was to 60% of the initial PAD amplitude and had a similar time course. We consistently failed to observe full recovery in the four experiments using bicuculline and in others using picrotoxin at the same concentration. However, in all of our experiments there was a gradual loss of signal amplitude with time, and this was accelerated by frequent changes of solutions in the saline chambers. Our interpretation of the apparently incomplete recovery in the bicuculline (and picrotoxin) experiments is that it is due to a gradual deterioration of the Vaseline seal separating the two saline-filled chambers of the sucrose gap. This decreases the gap resistance and, therefore, also reduces voltages recorded differentially across the gap.

GABA was applied iontophoretically (see METHODS). The microelectrode containing GABA was advanced along a track aimed at the reconstructed location of the fourth-root axons in the dorsal part of the central neuropil of ganglion VI (6), while test PADs were repetitively produced by giant-axon stimulation. Iontophoretic current pulses were delivered at a given depth, and the microelectrode was then advanced further. In all experiments, locations judged to be within the margins of the neuropil inhibited spontaneous activity levels, as recorded by a suction electrode on the five-six connective. Often this was the only effect observed; but on some tracks, when the microelectrode was judged to be near the zone of the fourth-root fibers, slow potential changes in the sucrose-gap record were produced by iontophoretic injection of GABA.

Figure 10 shows records from such an experiment. Giant-axon stimulation at 1 Hz evokes depolarizing PAD (bottom trace); the middle trace indicates the onset of the iontophoretic current. Interneuronal activity (upper trace) was inhibited during and after GABA release, with peak inhibition occurring about 1.5 s after current onset. In addition, a slow depolarization occurred in the sucrose-gap record within a few hundred milliseconds of current onset, peaking about 2.5 s, and then falling with a similar time course. During the depolarization, the evoked PAD was decreased. It is not possible to tell whether the decrease occurred because of receptor desensitization or because the membrane potential of the afferents had been reduced to the equilibrium potential for PAD. With this electrode placement, the time course for inhibition of spontaneous interneuronal
activity was faster than the time course of PAD, suggesting that we were nearer to sites of postsynaptic inhibition. At other sites, it was possible to produce PAD with little effect on spontaneous activity. Such results are to be expected if the sites for presynaptic and postsynaptic inhibition are spatially separated. In the experiment shown, depth measurements were taken and it was possible to return to the site producing depolarizations repeatedly; it appeared to be a more localized site than that responsible for the postsynaptic effects. Control experiments using NaCl were done on all preparations (see METHODS), and no effects were ever observed.

On some occasions, the sucrose-gap response recorded from afferents as a result of giant-axon stimulation has been of opposite sign, consistent with a hyperpolarizing rather than a depolarizing potential change in the afferents. We cannot explain these shifts, which sometimes take place during the first hour of a single experiment. In occasional preparations, responses were hyperpolarizing from the outset. However, reversed responses were more common in preparations in which the sixth ganglion was desheathed, suggesting that they may be associated with alterations in the ionic composition of the extracellular compartment surrounding the afferents. When GABA was injected into a preparation in which giant axon-evoked PAD was hyperpolarizing, the GABA response was also hyperpolarizing. In all other respects it was identical to those produced in normal preparations. This result strongly suggests that the response to GABA injection is mediated by the same conductance change that results from giant fiber stimulation.

**Ionic basis of PAD**

We suspected that PAD was chloride mediated because GABA produces chloride-selective conductance increases at all other junctions in Crustacea where its role has been evaluated (3, 22, 31, 32).

In earlier experiments (16) the reversal potential for PAD was estimated as lying between 12 and 20 mV, positive with respect to resting potential. We have confirmed these earlier observations; Fig. 11 shows the relationship between apparent membrane potential, which was shifted by current injected through the recording electrode, and the amplitude of PAD evoked by giant-axon impulses. The line is a least-squares fit of all points to the left of the

![FIG. 11.](image)

**FIG. 11.** Relationship between PAD amplitude and apparent membrane potential, obtained by current injection through a bridge. The line plots the linear regression for all points below +10 mV. Above this value there is substantial rectification of the electrode. The slope (m) and y intercept (b) were used to calculate the x intercept (c) according to the equation \( c = -\frac{b}{m} \). Filled circles represent a single data point; open circles indicate two overlapping values.
10 mV depolarization since attempts to pass larger currents caused substantial rectification of the electrode. The extrapolated reversal potential in this experiment was 14.5 mV depolarized with respect to resting potential, which is not consistent with either a sodium or a potassium equilibrium potential (although it could be due to a combination of the two conductances).

These experiments were all conducted using KCl electrodes. If chloride is the major ion involved in PAD, then the observed equilibrium potential would be expected to shift to increasingly depolarized levels as Cl\(^{-}\) leaks from the electrode and increases the internal chloride concentration. Evidence suggesting such shifts is shown in Fig. 12, where the reversal potential for PAD has become less negative than spike threshold, so that impulses are generated by summed PAD. Consistent with this finding, reductions of external [Cl\(^{-}\)] in sucrose-gap experiments also resulted in the generation of impulses from summed PAD.

Because we are working with a polysynaptic system, our attempts to clarify the ionic basis of PAD are subject to alternative interpretations. Nevertheless, all evidence is consistent with the hypothesis that Cl\(^{-}\) is the major ion involved in the conductance change underlying PAD.

**DISCUSSION**

**Generality of presynaptic inhibition**

Our observations, together with prior studies of this system (4, 5, 16, 17) provide a basis for comparing presynaptic inhibition in the CNS of crayfish and vertebrates. Six parallels emerge: 1) In both the crayfish and vertebrate CNS, presynaptic inhibition appears to be directed mainly at primary afferents (11, 27). 2) In both, presynaptic inhibition is in series with postsynaptic inhibition (11). 3) In both, presynaptic inhibition has a long time course, outlasting postsynaptic inhibition by tens to hundreds of milliseconds (12, 26). 4) In both, PAD accompanies inhibition. 5) For both, GABA is the putative transmitter (19, 20, and references therein). 6) In both crayfish and vertebrates, presynaptic inhibition is antagonized by bicuculline and picrotoxin, but not strychnine (13, 21, 27). A possible difference is that presynaptic and postsynaptic inhibition in vertebrates are mediated by separate inhibitory interneurons using different transmitters (11), whereas our evidence in the crayfish suggests that a common set of interneurons mediates both effects.

The large number of parallels between presynaptic inhibition of afferents across such diverse species as crayfish, frog, and cat is striking. In fact, it now appears that central presynaptic inhibition in Crustacea may have more in common with presynaptic inhibition in vertebrates than it has with presynaptic inhibition in the crustacean periphery. There, although probably mediated by the same transmitter, the ionic mechanisms differ (14), inhibition is very brief, lasting only about 6 ms (10), and it is associated with a hyperpolarization rather than a depolarization (14; see also Ref. 28).

**Features of circuit mediating presynaptic inhibition**

We have little information about the final inhibitory interneurons mediating presynaptic inhibition in response to giant-fiber stimulation. However, the pathway to them from the giant fibers is surely polysynaptic, judging from both the latency of PAD following giant-fiber stimulation (Fig. 5) and the PAD amplitude augmentation seen during high-frequency giant-fiber stimulation (Fig. 7). This augmentation may be meaningful in the normal function of the escape circuit; although single giant-fiber action potentials are sufficient to produce the entire sequence of events leading to

![FIG. 12. Example of PAD with a reversal potential less negative than spike threshold during intracellular recording with a KCl electrode. Intracellular record from an afferent fiber showing the largest summed PAD observed during the course of these studies (approximately 16 mV) and antidromic impulses evoked by PAD. The train of action potentials in the medial giant evoking this response is shown in the upper trace.](image-url)
escape, it is known from experiments with intact animals that the giant fibers can often discharge in brief bursts of from two to four impulses at a frequency of between 100 and 500 Hz (38). The limited number of PAD components we have been able to observe suggests that the final inhibitory elements are few in number, and results like those shown in Fig. 6 indicate that some of these final cells are interganglionic. However, evidence of the sort presented in Fig. 6 proved extremely difficult to obtain; it may be that most of the final inhibitory neurons are intraganglionic local circuit elements and are, therefore, inaccessible to experiments in which axons are isolated from the interganglionic connectives.

Although the number of final neurons producing presynaptic inhibition appears to be small, a variety of pathways have access to them. For example, we have found that presynaptic inhibition of afferents can be evoked by stimulation of either giant fiber, by the stimulation of any root containing mechanosensory afferents (16), or by stimulation of proprioceptive interneurons running in the ventromedial portion of the interganglionic connective (unpublished observations). The existence of a “final common path” class of interneurons seems likely because in every case so far studied there is occlusion of the PAD produced by different inhibitory pathways. However, our experiments indicate that the giant axons may act on these final inhibitory neurons through undefined but separate preinhibitory interneurons. This is suggested by the observation that single and multiple impulses in LG and MG produced PAD that differed in amplitude, latency, and component structure (Figs. 5, 8). It seems unlikely that the giant-axon synapses themselves have different integrative properties since all known excitatory contacts they make are electrical. Augmentation of GF-PAD is most likely due to the recruitment of final-path inhibitory neurons because unitary PAD components triggered by other pathways (unpublished observations) show no tendency to facilitate or depress at high stimulation frequencies.

The outputs of this small set of final inhibitory interneurons appear to distribute presynaptic inhibition widely among the mechanosensory afferents of the sixth ganglion. This conclusion is inferred from experiments in which PAD was recorded from single afferents with microelectrodes while recording the responses of large numbers of afferents with sucrose gap (Fig. 4). In every case where we observed a component of PAD being added to the population, we also saw the component in the intracellular record from the solitary afferent.

A particularly interesting aspect of the circuitry is that it imposes a delay of about 7–10 ms between the giant-axon’s impulse and the onset of inhibition. This delay occurs for both presynaptic and postsynaptic inhibition as well as for PAD and is consistent across all types of measurement (4, 5, 16, 17; Figs. 4, 5, 8). The functional significance of this delay is to make the moment of maximal inhibition (peak PAD amplitude) occur at about the same time (approximately 15 ms) as the maximum fast flexor muscle tension during a giant-fiber-triggered tail flip (see Ref. 37, Fig. 11). The same giant-axon impulses that cause delayed inhibition of the afferents evoke short-latency inhibition of extensor motoneurons (37), the motor giants (39), and of the giant axons themselves (24). Hence, the delay in command-derived inhibition is probably a built-in feature that matches the timing of inhibition to the timing of movement-induced reafference in mechanosensory systems.

Synaptic basis for PAD

Our evidence suggests that PAD is produced by a GABA-mediated increase in chloride conductance. This conclusion, though tentative, extends a generality: that GABA is either the established or the putative transmitter at every Crustacean inhibitory synapse at which it has been assessed. Chloride conductance increases mediate inhibition in crayfish sensory neurons (15), motoneurons (22), and muscles (32). The depolarization accompanying the chloride-conductance increase requires that chloride leave the afferents during PAD; it therefore must ordinarily be concentrated internally beyond its electrochemical equilibrium. Nothing is known about the chloride concentration in the afferent axons we studied, but Wallin (34) has shown
that the internal chloride concentration of crayfish giant axons is approximately 60% greater than would be predicted at equilibrium. These giant axons also have depolarizing ISPSs (24); those in the motor giant have an average reversal potential of $-53 \pm 5$ mV in cells with a resting potential of $-62$ mV (22).

Relationship between presynaptic and postsynaptic inhibition

An important point not fully resolved by these experiments is whether the same inhibitory interneurons mediate both presynaptic inhibition of afferents and postsynaptic inhibition of interneurons. It is known that both presynaptic and postsynaptic inhibition are produced by the same neuron in the periphery of the claw motor system (10), and results like those in Fig. 6, in which both forms of inhibition have identical latencies and identical patterns of component fluctuation, suggest that a common interneuron either makes the final synapses or is tightly coupled to the final interneurons.

However, the correspondence between presynaptic and postsynaptic inhibition is not complete, for presynaptic inhibition (measured by duration of tonic inhibition and protection) greatly outlasts postsynaptic inhibition (4, 5, 17). This result does not preclude a common final interneuron: the time course of the synaptic conductance increase could be determined postsynaptically or the presynaptic terminals of the interneuron could have varying location-dependent properties similar to peripheral synapses in the crayfish (2). Nevertheless, it does raise a functional question: if presynaptic and postsynaptic inhibition are inseparable, why should their time courses differ?

Mechanism of presynaptic inhibition

We stress that except for Figs. 1 and 2, our results deal principally with PAD, not with the presynaptic inhibition mechanism itself. The proposal that PAD is the electrophysiological correlate of presynaptic inhibition is reasonable inasmuch as there is complete correspondence between the pathways that produce PAD and those that produce presynaptic inhibition. On the other hand, it cannot be claimed that depolarization per se is a necessary condition for inhibition. Although PAD and inhibition both begin and peak at the same time, PAD is always considerably shorter than the inhibitory effect. Intracellular records of PAD have time courses ranging between 30 and 50 ms, while those recorded using the sucrose-gap method are between 50 and 80 ms. The discrepancy might be due to local damage of afferents penetrated by intracellular electrodes that shunt synaptic voltage changes. Another possibility is that sucrose-gap-recorded PAD, which represents the sum of PAD in all afferents that span the gap, allows one to see PAD in classes of afferents that have not been penetrated intracellularly. Total inhibition, as measured by the inhibition of EPSPs in sensory interneurons or protection against synaptic depression, can last up to 150 ms (4, 5, 16), but there is strong reason to believe that we may not be able to detect PAD in the majority of fourth-root afferents as these are less than 2 pm in diameter and are likely to have very short length constants.

There is no evidence for a conductance increase that outlasts PAD. Neither shifts in the membrane potential nor action potential shunting experiments (16; Fig. 3) reveal a prolonged conductance increase. We do not understand the discrepancy between the duration of PAD and presynaptic inhibition. A similar lack of temporal correspondence can be seen in records from spinal afferents and interneurons (11) and if the discrepancy can be firmly substantiated, it will have an important bearing on possible mechanisms of presynaptic inhibition.

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