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PRESYNAPTIC INHIBITION: THE MECHANISM OF PROTECTION FROM HABITUATION OF THE CRAYFISH LATERAL GIANT FIBRE ESCAPE RESPONSE

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SUMMARY

1. The mechanism of protection from habituation of the lateral giant escape reflex of the crayfish was studied. Experiments were designed to determine whether presynaptic inhibition of primary afferents for the reflex occurs following escape command neurone firing, and if so, whether it could account for protection of the first synapse from depression.

2. Synaptic transmission between afferents and interneurone A of the escape reflex is strongly inhibited following giant fibre spikes.

3. Giant fibre firing results in post-synaptic inhibition of interneurone A. However, inhibition of afferent input to interneurone A consistently outlasts both i.p.s.p.s and post-synaptic conductance increases in the neurone; the inhibition, therefore, is probably not exclusively post-synaptic.

4. Giant fibre firing results in excitability changes in sensory afferent terminals as measured by the amplitude of antidromic compound action potentials to focal electrical stimuli applied in the region of afferent terminals in the last abdominal ganglion. The time course of this effect parallels those of protection and inhibition of the first synapse.

5. The magnitude and time course of protection and inhibition of transmission to interneurone A parallel each other closely. Both processes considerably outlast measurable signs of post-synaptic inhibition.

6. We conclude that following giant fibre activity the first synapse of the lateral giant reflex is presynaptically inhibited, and the presynaptic inhibition is responsible for the protection effect described in the preceding paper.

INTRODUCTION

In the previous paper (Bryan & Krasne, 1977*a*) we showed that trains of stimuli which would normally cause habituation of the crayfish lateral giant fibre tail-flip escape reflex do not do so if the stimuli are applied following the firing of a tail-flip command neurone. It was argued there that the function of this protection from habituation is to prevent the escape reflex from becoming habituated to stimuli produced by an animal's own movements. Habituation in this system is apparently due to a reduction in transmission efficacy ('depression') at the first synapse of the escape reflex pathway (Zucker, 1972*b*), and it is the development of this depression from which the animal is protected during escape.

Most available evidence supports the view that synaptic depression of the sort studied here (e.g. Betz, 1970; Castellucci & Kandel, 1974; Farel, Glanzman & Thompson, 1973; Horn & Rowell, 1968; Thies, 1965; Zucker, 1972*b*), as well as other forms of synaptic plasticity, such as facilitation and post-tetanic potentiation (reviewed in Eccles, 1964, and Lang & Atwood, 1973), probably result from alterations in release of transmitter from presynaptic terminals.

Although the precise nature of the underlying presynaptic changes is not yet understood, in the case of synaptic depression there are several possible causes such as transmitter depletion (e.g. Betz, 1970; Castellucci & Kandel, 1974; Krasne, 1974; Thies, 1975; Zucker, 1972*b*) or alterations of terminal properties due to calcium ion accumulation (see, for example, Jansen & Nicholls, 1973; Meech, 1972) which one would expect to be reduced if the amplitude of action potentials in the presynaptic terminals were decreased, as is believed to occur during presynaptic inhibition (e.g. Eccles, 1964; Schmitt, 1971; Jahromi & Atwood, 1974). This line of reasoning suggested to us the possibility that protection from synaptic depression in the lateral giant escape reflex of the crayfish might be accomplished by presynaptic inhibition at the first synapse. Moreover, we noted, as discussed in the preceding paper, that the same command neurone firing which puts afferent terminals into a state where they are protected from depression also activates a powerful inhibition of the escape reflex pathway – an observation consistent with an important role for inhibitory processes in the protection phenomenon.

The experiments reported here were thus undertaken to determine whether the tactile afferent terminals of the escape reflex are presynaptically inhibited following command neurone firing and, if so, whether such presynaptic inhibition could account for protection of the first synapse from reductions in efficacy. A preliminary report of some of these results has been published previously (Krasne & Bryan, 1973).

METHODS

All experiments were carried out on isolated abdominal nerve cords of male and female crayfish, *Procambarus clarkii*. Dissection procedures, as well as procedures for stimulation and recording are described in the previous paper (Bryan & Krasne, 1977).

RESULTS

Relationship between protection and inhibition of transmission to lateral giants

Following the firing of a tail-flip command neurone, transmission between sensory afferents and the lateral giant escape command fibres is both protected from habituation *and* strongly inhibited (compare traces A_1 and

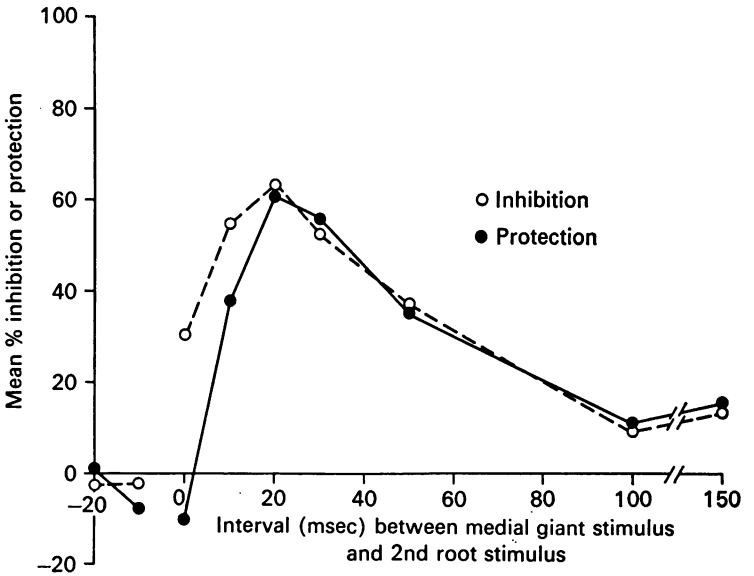

Fig. 1. Mean % inhibition or protection of e.p.s.p.s in the lateral giant fibre to second root stimulation as a function of the interval between a medial giant fibre stimulus and a second root stimulus; the medial giant spike arrived at the impaled ganglion 1–3 msec after the medial giant stimulus. Available data from five experiments were pooled (see Table 1). At negative intervals, the medial giant stimulus *followed* the second root stimulus.

B_1 of Fig. 1, Bryan & Krasne, 1977). The time courses of the protection and inhibition are compared in Fig. 1, based on data from five separate preparations. Not all intervals were tested in each preparation; therefore, not all experiments are represented at each point (see Table 1 for a breakdown of data by experiment). Mean percent inhibition (open circles) and

TABLE 1. Time course of inhibition of compound e.p.s.p.s and protection at the lateral giant fibre

Expt.	Mean	Latency (msec) from medial giant stimulus to second root stimulus								
		−20	−10	0	10	20	30	50	100	150
J9	Inhibition (%)	—	—	—	—	72·5 ($n = 4$)	71·0 ($n = 1$)	44·1 ($n = 1$)	8·0 ($n = 2$)	—
	Protection (%)	—	—	—	—	62·7 ($n = 4$)	47·8 ($n = 1$)	5·5 ($n = 3$)	7·7 ($n = 2$)	—
J11	Inhibition (%)	—	—	—	27·5 ($n = 2$)	49·7 ($n = 5$)	34·3 ($n = 2$)	9·3 ($n = 2$)	2·7 ($n = 2$)	—
	Protection (%)	—	—	—	13·5 ($n = 2$)	54·7 ($n = 5$)	63·1 ($n = 2$)	23·7 ($n = 2$)	12·4 ($n = 2$)	—
J13	Inhibition (%)	—	—	—	67·3 ($n = 3$)	67·3 ($n = 3$)	—	53·1 ($n = 3$)	15·7 ($n = 2$)	13·9 ($n = 2$)
	Protection (%)	—	—	—	39·1 ($n = 3$)	62·9 ($n = 3$)	—	71·7 ($n = 3$)	11·7 ($n = 2$)	13·7 ($n = 2$)
J15	Inhibition (%)	−2·4 ($n = 2$)	−0·8 ($n = 2$)	49·3 ($n = 3$)	70·2 ($n = 2$)	69·5 ($n = 2$)	—	—	—	—
	Protection (%)	0·7 ($n = 2$)	9·1 ($n = 2$)	−5·3 ($n = 3$)	63·5 ($n = 2$)	62·1 ($n = 2$)	—	—	—	—
J17	Inhibition (%)	—	−3·1 ($n = 4$)	1·2 ($n = 4$)	—	—	—	—	—	—
	Protection (%)	—	−24·9 ($n = 4$)	−17·0 ($n = 4$)	—	—	—	—	—	—

—, interval not tested in a given experiment.
n, number of runs.

mean percent protection (filled circles) were calculated using the individual data points for all experiments in which testing occurred at a particular interstimulus interval. Procedures for determining percent protection are described in Bryan & Krasne (1977); percent inhibition is defined as $[1-(\text{inhibited e.p.s.p./control e.p.s.p.})] \times 100$. Both mean % protection and mean % inhibition are maximal (approximately 60 %) when sensory afferent stimuli occur 20 msec after a medial giant spike. At the 50 msec interstimulus interval both effects are at roughly 50 % of their maximum levels, and at 100 msec are at roughly 20 % of their maximum levels. The marked correspondence between both the time course and the magnitude of the two processes, especially at interstimulus intervals greater than about 20 msec, suggests a causal relationship between protection and inhibition of transmission through the afferent limb of the reflex arc.

The inhibition observed in recordings from the lateral giant is, of course, the net effect of all inhibitory influences exerted upon the afferent limb of the reflex arc, including post-synaptic inhibition of the lateral giant (Roberts, 1968), as well as inhibition at the first synapse (described below).

It is quite unlikely that post-synaptic inhibition of the lateral giant could have any effect on the synaptic depression processes which are believed to occur exclusively at the first synapse (Krasne, 1969; Zucker, 1972*b*). Moreover, the time course of post-synaptic inhibition of the LG correlates poorly with the time courses of protection and over-all inhibition. It starts before protection begins (at 2–5 msec after the arrival of a medial or lateral giant spike) and terminates (usually by 70–90 msec) before protection or over-all inhibition of transmission to the lateral giant have concluded. (It is probably the short-latency onset of this post-synaptic inhibition that is responsible for the imperfect correspondence between the magnitudes of inhibition and protection at short interstimulus intervals – the 0 and 10 msec points in Fig. 1 and Table 1.)

Excitation and inhibition at the first synapse

The existence of inhibition at the first synapse following tail-flip command neurone activity is a logical consequence of the above observations and was observed in interneurone A in the previous paper (compare traces B_1 and B_2 of Fig. 6, Bryan & Krasne, 1977). As outlined in the introduction to this paper, we anticipated the existence of inhibition but expected it to be presynaptic. In fact, however, the firing of a tail-flip command neurone produces a diphasic post-synaptic potential (p.s.p.), a depolarization followed by a hyperpolarization, in interneurone A. Occasionally, we have seen the hyperpolarizing phase in the absence of the depolarizing

phase. When monosynaptic excitatory post-synaptic potentials (e.p.s.p.s) in the interneurone (Fig. 2*A*) are timed to occur at different latencies along the giant fibre-elicited p.s.p. (Fig. 2*C*), one can show that the initial depolarizing component is excitatory, and at very short interstimulus intervals can sum with a subthreshold e.p.s.p. to produce a spike (not shown in the Figure) while the later hyperpolarizing component is an

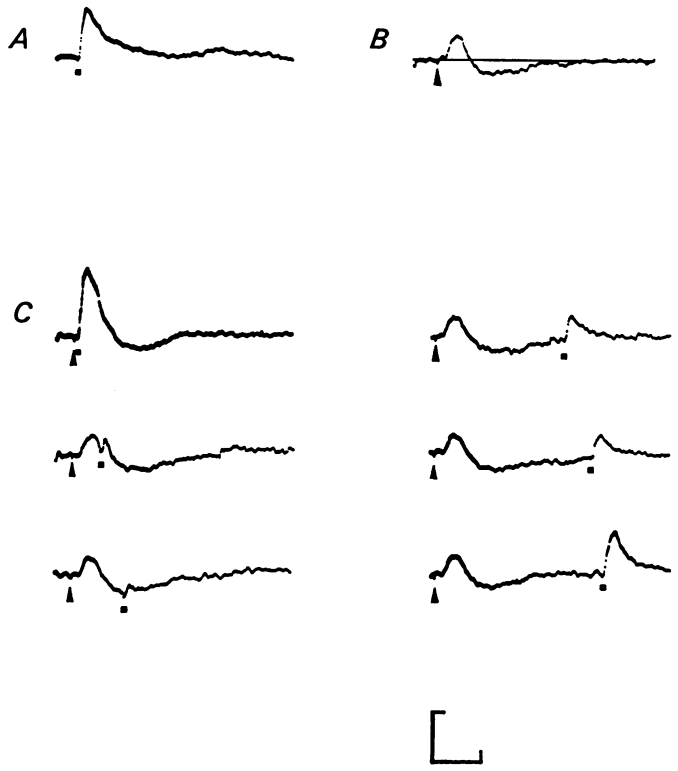

Fig. 2. Intracellular recording from interneurone A impaled just rostral to the last abdominal ganglion. *A*, monosynaptic compound e.p.s.p. to electrical stimulation (at square) of the ipsilateral first root. *B*, stimulation of cord giant fibres (at triangle) resulted in a biphasic post-synaptic potential in the interneurone. Resting membrane potential superimposed for comparison. *C*, interaction of first root stimulation (at square) with giant fibre stimulation (at triangle) at progressively longer interstimulus intervals. Vertical calibration, 2 mV; horizontal calibration, 20 msec.

inhibitory post-synaptic potential (i.p.s.p.) and is associated with a large reduction in the amplitude of appropriately timed e.p.s.p.s. The initial e.p.s.p. has been observed in intracellular recordings from the interneuronein response to focal stimulation of both lateral and medial giants,

and we have noted in extracellular experiments that a subthreshold sensory root stimulus given at the proper time following medial giant firing causes interneurone A to fire. We therefore believe that both the medial and the lateral giant can evoke the depolarization. Similarly, the i.p.s.p. follows firing in either escape command cell.

Relation of the i.p.s.p. to inhibition at the first synapse. Careful measurements of the duration of inhibition and of i.p.s.p.s were made in eight preparations (Table 2). In each preparation inhibition outlasted the mean i.p.s.p. in that preparation by 15–90 msec, and the longest i.p.s.p. seen in that preparation by 15–75 msec. In order to compare the time courses of the i.p.s.p. and inhibition graphically, the mean hyperpolarization and inhibition were calculated for each preparation in 15 msec time bins, and then each animal's means were normalized relative to the values taken in the 16–30 msec bin. A plot of the means of these normalized values (Fig. 3) shows clearly that inhibition considerably outlasts the i.p.s.p. This is most conspicuous in the 70–100 msec time range. At 70 msec the mean hyperpolarization is essentially zero, while inhibition is still at 35 % of its maximum value and does not seem to return fully to base line until sometime after 160 msec (though these late values are not statistically reliable).

Thus, inhibition occurs at times when there is no indication from the membrane potential that there is any post-synaptic inhibition operating. This suggests the existence of the presynaptic inhibition that we had expected.

Post-synaptic conductance measurements during inhibition. Although the return of the i.p.s.p. to base line at 70 msec indicates that the conductance change responsible for *it* is probably complete by 70 msec, there could also be components of post-synaptic inhibition that produce longer-lasting conductance increases that hold the membrane very near the resting level thereby accounting for some of the longevity that we have suggested might be of presynaptic origin. This possibility was investigated by determining the amount of shunting of antidromic axon spikes in interneurone A at a dendritic recording site.

Penetration of presumed dendritic processes was facilitated by maps of interneurone A derived from Procion yellow (Selverston & Kennedy, 1969) and our own cobalt (Pitman, Tweedle & Cohen, 1972) injections. A dendrite was identified on the basis of its antidromic response to focal stimulation of the cell's axon by a suction electrode on the ventrolateral surface of the 5/6 abdominal connective. While intracellular axon spikes were 90–110 mV and over-shooting, the spike heights measured at the presumed dendritic location were 44–65 mV and not over-shooting. Apparent critical firing levels at the latter site were also large (20–25 mV) compared to those at the main axon (typically less than 8 mV). These observations are consistent

TABLE 2. Inhibition and protection at interneurone A following activation of cord giant fibres

Expt.	Mean	Latency (msec) from giant fibre stimulus to afferent root stimulus											
		0–15	16–30	31–45	46–60	61–75	76–90	91–105	106–120	121–135	136–150	151–165	300
J34	I.p.s.p.		T_m			T_x, T_L							
	Inhibition (%)	—	86·0 ($n = 1$)	78·7 ($n = 2$)	49·9 ($n = 3$)	34·6 ($n = 4$)	17·9 ($n = 7$)	—	—	—	—	—	—
	Protection (%)	—	69·3 ($n = 3$)	—	—	29·3 ($n = 2$)	35·9 ($n = 1$)	—	—	—	—	—	—
J40	I.p.s.p.		T_m		T_x	T_L							
	Inhibition (%)	—	76·2 ($n = 3$)	—	36·0 ($n = 4$)	—	—	15·8 ($n = 4$)	—	—	—	—	0·8 ($n = 3$)
	Protection (%)	—	100·0 ($n = 2$)	—	100·0 ($n = 1$)	20·9 ($n = 1$)	—	15·3 ($n = 1$)	—				
J41	I.p.s.p.		T_m			T_x	T_L						
	Inhibition (%)	—	95·4 ($n = 2$)	—	84·7 ($n = 2$)	—	39·5 ($n = 3$)	—	7·8 ($n = 3$)				
	Protection (%)	—	59·9 ($n = 2$)	—	52·7 ($n = 2$)	—	10·9 ($n = 3$)	—	8·5* ($n = 2$)	—	—	—	—
J42	I.p.s.p.			T_m			T_x, T_L						
	Inhibition (%)	—	94·3 ($n = 3$)	—	—	—	—	25·4 ($n = 2$)	—	—	—	—	—
	Protection (%)	—	86·9 ($n = 3$)	—	—	—	—	41·0 ($n = 2$)	—	—	—	—	—
J43	I.p.s.p.			T_m		T_x	T_L						
	Inhibition (%)	—	96·3 ($n = 3$)	—	70·0 ($n = 1$	45·0 ($n = 2$)	—	12·5 ($n = 1$)	4·9 ($n = 1$)	—	—	—	—

TABLE 2 (*cont.*)

Expt.	Mean	Latency (msec) from giant fibre stimulus to afferent root stimulus											
		0-15	16–30	31–45	46–60	61–75	76–90	91–105	106–120	121–135	136–150	151–165	300
	Protection (%)	—	86·8 ($n = 3$)	—	60·0 ($n = 1$)	30·3 ($n = 2$)	—	−7·3 ($n = 1$)	3·5 ($n = 1$)	—	—	—	—
J35	I.p.s.p.		T_m		T_x	T_L							
	Inhibition (%)	38·6 ($n = 1$)	75·3 ($n = 2$)	32·1 ($n = 2$)	36·0 ($n = 4$)	14·5 ($n = 3$)	13·7 ($n = 4$)	9·6 ($n = 2$)	8·3 ($n = 4$)	6·3 ($n = 2$)	3·1 ($n = 2$)	—	—
J36	I.p.s.p.		T_m				T_x	T_L					
	Inhibition (%)	—	95·2 ($n = 1$)	68·6 ($n = 1$)	—	37·0 ($n = 1$)	34·5 ($n = 1$)	26·6 ($n = 1$)	15·0 ($n = 3$)	—	—	13·0 ($n = 1$)	—
J38	I.p.s.p.		T_m		T_x		T_L						
	Inhibition (%)	87·4 ($n = 6$)	77·3 ($n = 2$)	—	46·8 ($n = 3$)	—	—	22·6 ($n = 3$)	15·1 ($n = 3$)	—	5·5 ($n = 3$)	—	—

I.p.s.p.: T_m = interval during which maximum hyperpolarization occurred; T_x = interval containing mean latency to i.p.s.p. repolarization; T_L = interval containing longest i.p.s.p.; —, data not obtained for that interval; n = number of runs. * One point (% protection = −43·2 %) omitted from mean; see Bryan & Krasne (1977) for reasons for omission.

with a recording site at some distance from the site of impulse initiation and indicate that action potentials do not actively propagate into the dendritic region of this neurone. In five of eight preparations in which a dendrite was impaled, the inhibitory portion of the post-synaptic potential produced by giant fibre stimulation was normal in its temporal characteristics, but reversed in sign (i.e. depolarizing). We do not know the reason for this; one possibility is leakage of Cl^- into the confined space of the impaled dendrite.

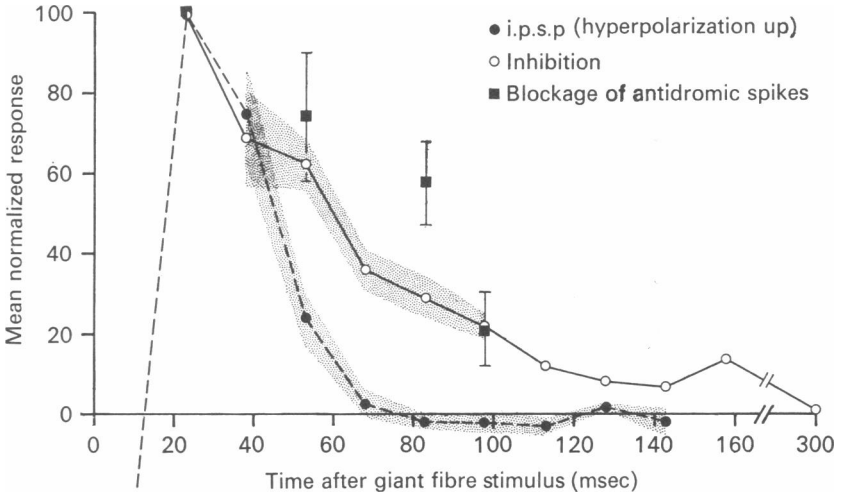

Fig. 3. The time course of hyperpolarization and inhibition at interneurone A and of blockage of antidromic spikes in afferents as a function of the delay (in msec) between giant fibre stimulation and the measurement. See text for explanation of measures graphed. Stippled variability markers are S.E. of means across animals (each animal providing one data point). Variability markers on 'blockage' measurements are S.E. of means of all measurements, pooled across preparations; 'blockage' measurements were not done on the same preparations as the hyperpolarization and inhibition measurements.

Changes in transmembrane conductance in the dendrites were observed by superimposing directly elicited action potentials in interneurone A upon the giant fibre-evoked i.p.s.p. at different interstimulus intervals and measuring changes in the amplitude of the antidromic spike at the dendritic recording site. Since antidromic spikes appear to be passively conducted to this recording site (see above), spike height should be relatively sensitive to increases in membrane conductance resulting from inhibitory transmitter action, provided that such inhibitory synaptic input is not too distal.

In Fig. 4 *A* 1, direct stimulation of the axon of interneurone A follows stimulation of cord giant fibres (at first long arrow) at successively longer

intervals. Traces of antidromic spikes occurring at different latencies after elicitation of the i.p.s.p. are photographically superimposed to illustrate the shunting effect. Antidromic spike amplitude has returned to its control (uninhibited) size by the sixth antidromic stimulus. In Fig. 4 *B* the magnitude of the shunting (open triangles) is plotted as a function of the time from giant fibre stimulation to antidromic stimulation of interneurone A. Shunting begins at 10 msec. Maximum shunting occurs approximately

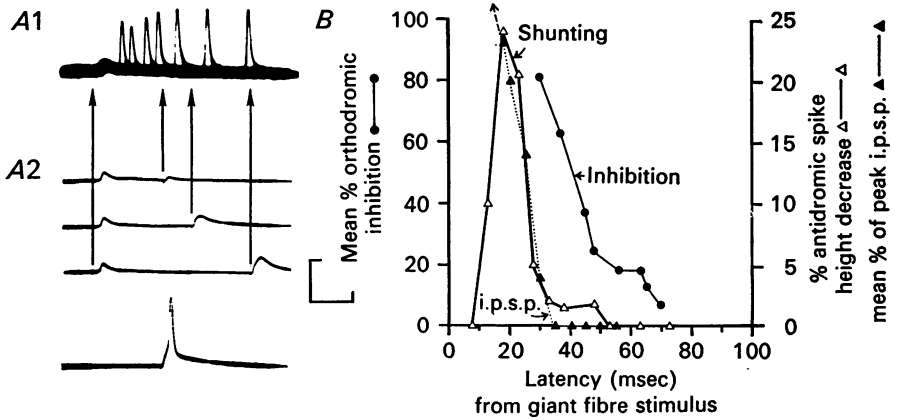

Fig. 4. Comparison of the time course of shunting of antidromic axon spikes with inhibition of orthodromically elicited e.p.s.p.s at a dendritic recording site in interneurone A. *A*1, photographic superimposition of antidromic axon spikes at various latencies along the giant fibre elicited post-synaptic potential. The last two spikes at the right are the same size as a control antidromic spike. *A*2, inhibition of orthodromic e.p.s.p.s in the same preparation following giant fibre stimulation. Bottom trace shows control response to the orthodromic stimulus alone. Vertical calibration: 40 mV; horizontal calibration: 20 msec. *B*, graphic comparison of decrease in antidromic spike height, e.p.s.p. inhibition, and depolarization (i.p.s.p.) in this preparation as a function of the latency from a giant fibre stimulus. The i.p.s.p. is normalized as % maximum depolarization (which occurred at the peak of the initial excitatory portion of the post-synaptic response). *A* and *B* are from the same preparation.

18 msec after direct activation of cord giant fibres and results in an approximately 24 % reduction in antidromic spike height at the dendritic recording site. Small (1–2 %) decreases in spike height occur as late as 44 msec after the giant fibre stimulus, but by 53 msec there is no further sign of the shunting. Fig. 4 *B* also indicates that the i.p.s.p. (filled triangles), normalized as % maximum depolarization, shows a close correspondence in time course with the conductance increase, though the latter may persist marginally for perhaps 20 msec longer. Both measures of post-synaptic inhibitory action are at zero by 53 msec. However, in this same

preparation inhibition of an orthodromically elicited action potential (Fig. 4 *A* 2), considerably outlasted post-synaptic inhibition. The bottom trace (Fig. 4 *A* 2) shows an orthodromic action potential set up by electrical stimulation of a first root. The top three traces of Fig. 4 *A* 2, show inhibition of the spike to an identical stimulus delivered at successively longer intervals after giant fibre stimulation. Comparison of traces in 4 *A* 1 and 4 *A* 2 illustrates that inhibition of synaptic input persists well beyond the post-synaptic dendritic inhibitory conductance increase. Mean % orthodromic inhibition (filled circles, Fig. 4 *B*) was tested at giant fibre-first root interstimulus intervals from 20 to 70 msec. At 56 msec, when there was no measurable post-synaptic inhibition, orthodromic input to interneurone A was still inhibited by nearly 20 %.

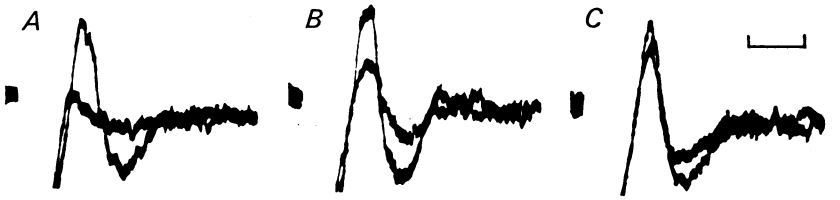

Fig. 5. Antidromic sensory root responses during inhibition. Compound action potentials in the fourth (sensory) root of the last abdominal ganglion were elicited by shocking the terminal region of the root via a 5 μm stainless-steel electrode in the neuropile. Antidromic stimuli were given (*A*) 20 msec, (*B*) 80 msec and (*C*) 100 msec after giant fibre stimulation. In each case a control response to antidromic stimulation alone (the larger response) is superimposed on the test response. Calibration: 1·0 msec.

The finding that, at the first synapse of the lateral giant reflex, inhibition of transmission consistently outlasted all signs of post-synaptic inhibition supports the hypothesis of presynaptic inhibition of the tactile sensory afferents for the reflex.

Excitability changes in sensory neurone terminals. If tactile sensory afferent terminals are presynaptically inhibited following giant fibre impulses, one would expect conductance increases and perhaps depolarizations of the terminals during inhibition. Such presynaptic inhibitory effects might alter the extent to which focal current passage near the terminals could evoke antidromic spikes in the axons of origin. This was tested for afferents entering over the fourth root of the last abdominal ganglion. This root was chosen because it is entirely sensory except for a single efferent neurone (Calabrese, 1976).

Neuropile about half-way laterally from the mid line of the sixth abdominal ganglion at the level of entrance of the first root, where the fourth root fibres overlap the dendritic arbor of interneurone A and where many

of them appear to terminate (Calabrese, 1976), was explored with 5–30 μm steel electrodes for points where we could evoke fourth root antidromic potentials that were: (i) gradable with stimulus current (which was then set for an intermediate-sized response), and (ii) subject to changes in size when evoked after the firing of a giant fibre. Fig. 5 shows compound action potentials in the fourth root of one such preparation. In traces *A–C* the compound antidromic response to a focal neuropile stimulus alone (larger response in each frame) is superimposed upon the antidromic response to a neuropile stimulus that is preceded by a giant fibre stimulus. The compound action potentials were changed in size when evoked after activation of giant command neurones. Most often decreases (as in Fig. 5), which we attribute to shunting effects of axon terminal conductance increases, were found.

At some focal stimulating electrode locations the compound antidromic spike increased following giant fibre firing. We presume that these were cases where the electrodes were sufficiently distant from the inhibitory synapses so that the depolarization spreading from the synapses (Kennedy, Calabrese & Wine, 1974) could lower antidromic firing threshold with minimal shunting effects.

The time course of these effects roughly paralleled that of protection (Figs. 5 and 6), with marked reduction of the antidromic response at 20 msec, and some reduction as late as 100 msec after giant fibre stimulation. In two preparations the percentage reduction in the peak-to-peak amplitude of the compound antidromic potential was calculated for potentials obtained at a range of times after giant fibre firing. These were normalized and averaged for plotting in Fig. 3. Percentages for each animal were normalized by dividing each percentage by the mean of all the values for that animal in the 16–30 msec time bin. Means and S.E. of means of the normalized data were taken across all available measurements in each of the 15 msec time bins.

The fact that the compound antidromic potential could be graded as a function of giant fibre firing-test shock interval rules out the possibility that the effects are due to the single fourth root efferent fibre.

Protection and presynaptic inhibition

The data presented above suggest very strongly the occurrence of presynaptic inhibition at the first synapse of the lateral giant reflex. If this presynaptic inhibition is responsible for protection from depression at this synaptic relay, then protection should follow the time course of inhibition of transmission at this synapse and outlast post-synaptic inhibition.

Data from five preparations in which both the time course of inhibition and protection were determined are presented in Fig. 6. The average time course of i.p.s.p.s (from Fig. 3) is included, normalized to 84 % at 24 msec

to facilitate comparison with the other curves. Both the time courses and the absolute magnitudes of the protection and inhibition curves parallel one another closely. Over the range of intervals examined, protection and inhibition are maximum in the 16–30 msec interval, and are still demonstrable at 106–120 msec, well after all signs of post-synaptic inhibition

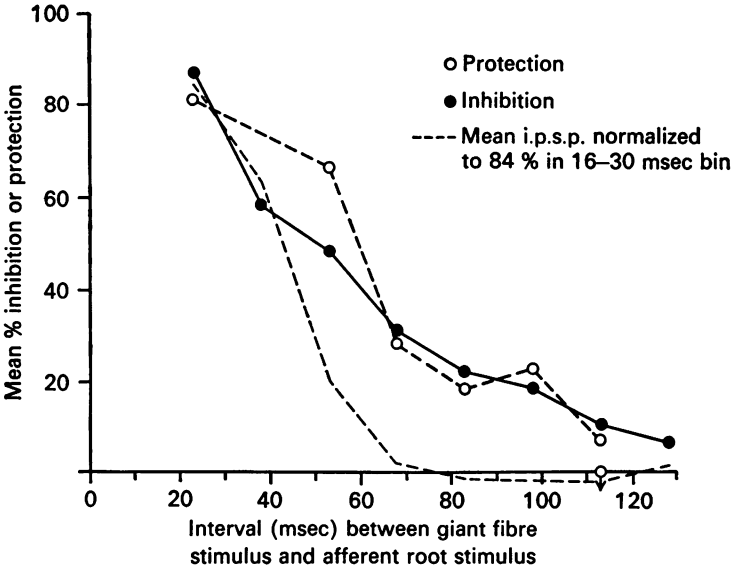

Fig. 6. Comparison of the time course of protection (open circles) and inhibition (filled circles) in interneurone A as a function of the interval between the giant fibre stimulus and the sensory root stimulus. Data from five preparations were pooled. Each point is the mean of individual observations from the experiments (see Table 2 for breakdown by experiment; data grouped as in Table 2). The average time course of i.p.s.p.s (interrupted curve; from Fig. 3) is included, normalized to 84 % at 24 msec to facilitate comparison.

have abated (see Bryan & Krasne (1977) for a discussion of the protection point plotted on the abscissa in this interval). The correspondence between protection and inhibition, and the persistence of both well beyond the apparent cessation of post-synaptic inhibition, supports the hypothesis that presynaptic inhibition is responsible for protection.

DISCUSSION

Presynaptic inhibition, post-synaptic inhibition, and protection

The evidence presented in the previous paper and above has led us to postulate the existence of presynaptic inhibitory synapses on tactile afferents of the lateral giant escape reflex (Fig. 7, pathway I) and to argue

that they function to protect the first synapse of the reflex from depression when such depression would be maladaptive.

Our case for presynaptic inhibition is compelling when all the evidence is taken together. Nevertheless, the evidence is indirect. The most direct evidence, that of alterations in afferent terminal excitability during the inhibition, is perhaps also the weakest, because we cannot rule out the

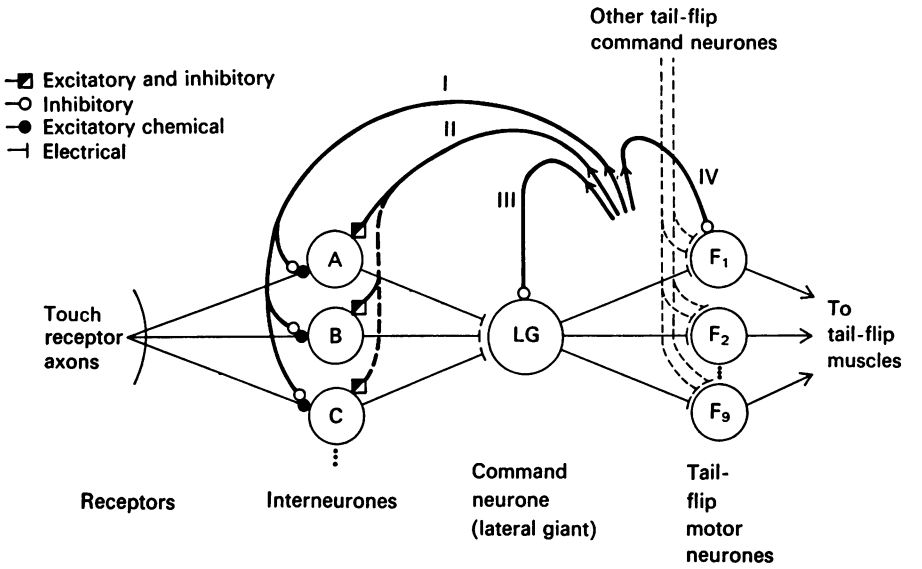

Fig. 7. Crayfish lateral giant (LG) escape reflex circuit showing feed-back pathways. The basic circuit (based on Zucker, Kennedy & Selverston, 1971) is indicated at the bottom of the figure. Feed-back pathways (specific neurones not yet identified) activated by giant fibres are indicated by bold pathways I-IV (see text). Dashed lines indicate circuitry conjectured on the basis of symmetry.

possibility that numerous post-synaptic conductance changes throughout the ganglion alter the current paths set up by our focal test shocks and thereby produce the effects that we see. Such criticisms, however, completely lose their force in the face of recent observations by Kennedy *et al.* (1974) that during the time when we have postulated the operation of presynaptic inhibition, fourth root afferents within the last abdominal ganglion are depolarized, and spikes arriving from the periphery are reduced in both baseline-to-peak amplitude and in absolute size at their peak. Such direct evidence for both absolute and relative reduction of presynaptic spike amplitude during presynaptic inhibition is in fact available in no other system, and taken together with our results makes the case for presynaptic inhibition very strong indeed.

One would expect presynaptic inhibition to cause protection from synaptic depression, since most of the candidate mechanisms of depression such as transmitter depletion, post-synaptic receptor desensitization, and presynaptic terminal conductance increases due to calcium accumulation should be attenuated by presynaptic inhibitory inputs that operate through reducing presynaptic terminal spike size, and thus probably also the extent of calcium entry and of transmitter release per trial.

Our data do not rule out the possibility that post-synaptic inhibition, which also occurs at the first synapse during tail-flips (Fig. 7, pathway II), might contribute to protection; but this seems very unlikely. Changes occurring presynaptically or in the steps whereby transmitter produces e.p.s.p.s would not be affected by post-synaptic inhibition unless one were to postulate exotic post-synaptic-to-presynaptic trophic effects or effects of post-synaptic cell spiking on synaptic efficacy (see e.g. Stent, 1973). In neurones whose response to excitatory transmitter involves dendritic branch spikes or non-propagating local electrogenic responses, apparent synaptic depression could in principle result from a diminution of these e.p.s.p.-evoked electrogenic events due to some sort of long-lasting refractoriness or accommodation. In such cases post-synaptic inhibition, which reduces e.p.s.p. size, could protect dendritic tree electrogenic mechanisms from excitation and therefore also from depression. However, tests for long-lasting refractoriness in first-order tactile interneurones of the lateral giant reflex that do have some active sub-threshold electrogenic responses (Calabrese & Kennedy, 1974) have proven negative (Zucker, 1972*a*). Even in systems where cumulative blockage of this sort has been seen, it has required frequencies in excess of 25–50 stimuli per second and lasted only tens of milliseconds (e.g. Zucker, 1972*b*). Moreover, there is no indication that active non-propagating electrogenic responses occur in the dendrites of interneurone A (Kennedy, 1971; see also Wine, 1975). Finally, almost all available evidence (but see Stephens, 1973*a*,*b*) is consistent with the view that synaptic depression is due to diminished release of transmitter rather than with later events.

Implications of protection for mechanisms of habituation

Unfortunately, presynaptic inhibition probably exerts its effect so early in the sequence of events responsible for synaptic transmission that the mere fact of protection provides little help in localizing causes of depression. One can, however, conclude that depression at these crayfish synapses is not due to changes in electrical excitability or in ion concentrations that result directly from repeated spiking of portions of the presynaptic axon that are proximal (relative to the soma) to the presynaptic inhibitory synapses.

It should be noted (Fig. 6 and see also Fig. 1 at intervals past 20 msec) that protection and inhibition of the protected synapse are about equal numerically over a wide range of values. This means that the drop in (uninhibited) e.p.s.p. size that occurs as a result of a protected habituation series is strictly proportional to the size of the (inhibited) e.p.s.p. on the first trial of the series. So long as inhibition is mostly presynaptic (which it is at late intervals) and reduces e.p.s.p. size by a fixed percentage that is independent of degree of habituation, this result is predicted by models of habituation or depression such as those of Thies (1965), Betz (1970), and Woodson, Schlapfer, Tremblay & Barondes (1976*a*) which assume that transmitter release on a trial is a constant fraction F of the transmitter remaining in an available pool that is continually refilled according to first-order reaction kinetics. However, other models can make similar predictions. For example, on the basis of experiments with altered Ca^{2+}/Mg^{2+} ratios Bruner & Kennedy (1970) suggested that synaptic depression at motor giant neurone-to-fast flexor muscle synapses in the escape reflex pathway may be due to propagation failures in motor neurone terminals. The precise equality of percentage inhibition and protection would follow from this hypothesis if presynaptic inhibition were to operate n an all-none fashion, fully blocking propagation into some terminal arbor branches (which would then not alter their properties during habituation) and permitting propagation into others.

Role of presynaptic inhibition in the escape reflex

We have concentrated in this paper on the presynaptic inhibition evoked by escape command neurone firing. However, this is but one out of a number of adjustments, mostly inhibitory, which are made to escape reflex circuitry after escape command neurones fire (Fig. 7, pathways I-IV). These include (1) a 10 msec period of enhanced transmission between sensory neurones and first-order tactile interneurones (or at least interneurone A) before both the onset of inhibition and of movement, (2) post-synaptic inhibition of first-order tactile interneurones starting, along with the presynaptic inhibition, at about 10 msec after giant fibre firing (and at or shortly before the moment when movement would be expected to begin), (3) post-synaptic inhibition of the lateral giant command neurone (and also of the medial giant) starting 2–4 msec after giant fibre firing, and thus before the inhibition at the earlier synapse (Roberts, 1968), and (4) post-synaptic inhibition of giant motor nuerones (but not the non-giant motor neurones) which feed tail-flip musculature at about 3 msec after command neurone firing (Hagiwara, 1958; Mittenthal & Wine, 1973; Roberts, 1968; J. J. Wine & F. B. Krasne, in preparation). Since this amounts to a pattern of rather wide-spread inhibition of the escape circuitry we should

question whether the functional significance of presynaptic inhibition is to protect the first synapse from decreased efficacy or whether protection is a fortuitous by-product of a general inhibition serving a different purpose. This is essentially a question about selection pressures, which are often multiple, so the answer may be that both hypotheses are correct. However, the non-uniform timing arrangements discussed above suggest that the different adjustments made to the escape reflex after giant fibre firing are not all meant to do the same thing. The initial period of facilitation at the first synapse is an interesting mystery; it suggests that the nervous system does something important with tactile sensory information which arrives shortly after an escape command neurone fires but before movement begins. The timing of the inhibition at the first synaptic relay suggests that it serves to nullify the consequences of input from self-produced movement. The post-synaptic component would tend to silence previously initiated after-discharge in the tactile interneurones once movement gets under way, which is a function *not* accomplished by presynaptic inhibition. The timing of the inhibition of the command neurone and the giant motor neurones, which comes well before movement starts, presumably serves to regulate the *number* of spikes produced by these neurones, a function quite different from that of the inhibitory influences at the first synapse.

Kennedy *et al.* (1974) have recently demonstrated that the same sort of pre- and post-synaptic inhibition of sensory afferent-interneurone synapses such as that described here can be produced by tactile sensory input that is below threshold for firing escape command neurones. In line with our findings, we would speculate that the presynaptic component might serve to prevent stimuli that are prolonged but too weak to fire escape command neurones from producing massive synaptic depression of tactile afferents due to repeated firing. It is also possible, however, that the tactile input which produces inhibition without firing *escape* command neurones does nevertheless produce some reflex movements from whose resultant stimuli the escape reflex must be protected.

Generality of protective effects of presynaptic inhibition

Our data relating protection from synaptic depression to presynaptic inhibition suggest a new role for presynaptic inhibition, namely that of reducing or preventing the occurrence of alterations in the transmission properties of synapses that exhibit plasticity to repeated stimulation.

Any form of use-induced change in synaptic efficacy that is dependent on magnitude of terminal depolarization will be likely to develop less fully if presynaptic spikes arrive during presynaptic inhibition. It is presumably because of this that the development of temporal facilitation at some crustacean neuromuscular junctions is diminished if motor neurone spikes

arrive at the synapse while it is presynaptically inhibited (Dudel & Kuffler, 1961; Atwood & Bittner, 1971; Wiens & Atwood, 1975). However, such effects are not always seen and are often quite small (e.g. Dudel & Kuffler, 1961; Kennedy, 1977; D.A. Baxter and G.D. Bittner, personal communication). Development of facilitation is known in some cases to be surprisingly insensitive to size of presynaptic spikes (Charlton & Bittner, 1977*a*,*b*), so protection from facilitation may be limited to cases where presynaptic inhibition blocks invasion of terminals almost totally. Functionally, it seems plausible that presynaptic as opposed to purely post-synaptic inhibition might be used at facilitating crustacean neuromuscular junctions in order to minimize the development of facilitation when transmission is inhibited. Those fibres within a muscle whose synapses are most prone to facilitation might then be expected to receive presynaptic inhibitory innervation preferentially; however, available evidence does not support this expectation (see Atwood & Bittner, 1971). Alternatively, Kennedy (1977) has argued that the function of presynaptic inhibition at facilitatable arthropod junctions might be to protect them from becoming depressed during build up of facilitation, a view suggested by the work of Marmont & Wiersma (1938).

Post-tetanic potentiation at synapses between visceropleural connective fibres and cell R15 of *Aplysia* abdominal ganglion is also diminished if spikes arrive following an inhibitory volly to the presynaptic elements of these junctions (Woodson *et al.* 1976*b*). However, since the effect of such a volley on post-tetanic potentiation greatly outlasts the inhibition of the e.p.s.p., it is not clear whether this phenomenon is comparable to the others under discussion here.

Whatever their function, a useful practical consequence of protection phenomena is that they suggest a convenient diagnostic criterion to aid in distinguishing between remote post-synaptic inhibition and presynaptic inhibition at synapses where some measure of depression, facilitation, or post-tetanic potentiation can be produced.

In the vertebrate nervous system presynaptic inhibition is believed to occur extensively at early relays (Schmidt, 1971). Phenomena such as visual and auditory adaptation (Green, Dowling, Siegel & Ripps, 1975; Furukawa & Ishii, 1967; Benitez, Eldredge & Templer, 1972), novelty detection in the dorsal horn (Wall, 1967), wind-up in the high threshold 'pain' system of the dorsal horn (Mendell, 1966), etc., suggest that early sensory synapses might possibly be subject to some alteration of efficacy with use. Thus, we believe that, as in the crayfish, presynaptic inhibition in vertebrate sensory pathways could serve a protective function. This suggestion has several times previously been made to provide a possible rationale for centrifugal inhibition of hair-cell to sensory neurone trans-

mission in the vertebrate acoustico-lateralis system (see Klinke & Galley, 1974; Flock, 1965; Russell, 1971; Roberts & Russell, 1972; Davis, 1968), but to our knowledge experimental tests of the notion have not been made.

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