EXPERIENTIA

Vol. 24 - Fasc. 8 Pag. 753–864 15. 8. 1968

Peripheral Inhibition in Crustacean Muscle

by H. L. ATWOOD

Department of Zoology, University of Toronto, Toronto (Canada)

The purpose of this article is to present a survey of what is presently known about the mechanisms of peripheral inhibition in the striated muscles of crustaceans.

The inhibitory neurons of crustacean muscles have undergone a great deal of experimental investigation since the observations of BIEDERMANN[1] and of HOFFMANN[2] first suggested their existence. The reasons for this interest are many. First, the accessibility and relatively large size of these neurons facilitates their isolation for physiological and biochemical analysis. Moreover, crustacean neuromuscular systems provide a simplified and accessible model for study of certain physiological activities (especially, integration of diverse synaptic inputs) which are also important in central nervous systems. Crustacean peripheral inhibitory neurons duplicate in some measure inhibitory phenomena in the vertebrate central nervous system. Other arthropods have recently been found to possess peripheral inhibitory neurons[3,4], but for reasons of size it is likely that crustaceans will continue to provide the favourite preparations for detailed analysis of the mechanisms of peripheral inhibition.

Organization of Crustacean Neuromuscular Systems

The crustacean neuromuscular systems differ in organization from those of vertebrates in 2 major respects: (a) there is a rather small number of efferent axons supplying a crustacean muscle, although each axon has a very large number of synapses; (b) the efferent axons are of 2 primary types, excitatory (E-axon) and inhibitory (I-axon). Activity of the latter type serves to weaken or completely suppress muscular contractions elicited by stimulation of the former. The extent of tension reduction depends on the frequencies of nerve impulses in the 2 types of axon[5].

Two examples of organization in crustacean neuromuscular systems are given in Figure 1, which shows the innervation patterns of muscles in the leg of a crab[5] and in the fast extensor muscles of the crayfish abdomen[6]. Each muscle receives 1–4 E-axons and 1 or 2 I-axons. Fibres within a muscle may be innervated by varying numbers of the axons supplying that muscle ([7]; Figure 2).

Two of the muscles in the crab leg, the 'opener' and the 'stretcher', are innervated by a single motor axon; each has dual inhibitory innervation. (In other decapods the inhibitory innervation of these muscles is supplied by 1 I-axon.) One of the I-axons of the crab (the 'common inhibitor') is distributed to at least 5 muscles, some of which are functional antagonists. In the crayfish abdomen, sharing of inhibitory and motor axons by separate muscles is also apparent, although in this case all muscles are functionally synergistic. Overlap of innervation seems to be a common feature in crustacean systems.

The number of synapses associated with each axon is large. For a single muscle fibre in the crayfish opener

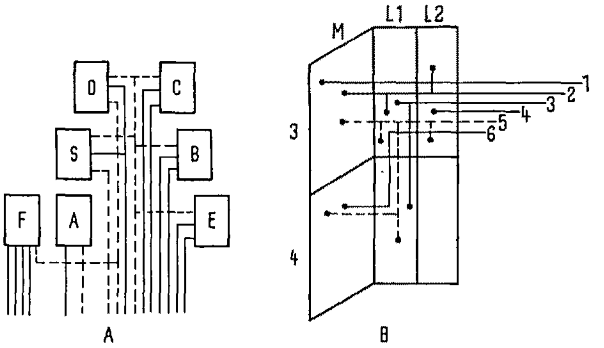

Fig. 1. Distribution of axons to muscles in a crab leg (A) and to the deep extensor muscles of the crayfish abdomen in segments 3 and 4 from the nerve to the third segment (B). Solid lines are motor axons; dotted lines are inhibitory axons. The muscles are: (A) opener or abductor of the dactylopodite, O; closer or abductor of the dactylopodite, C; stretcher, S; bender, B; extensor of the carpopodite, E; flexor of the carpopodite, F; accessory flexor, A; (B) medial deep extensor muscles, M; lateral deep extensor muscles, L_1 and L_2. (After WIERSMA[5] and PARNAS and ATWOOD[6].)

---

[1] W. BIEDERMANN, Sber. Akad. Wiss. Wien III *96*, 8 (1887).

[2] P. HOFFMANN, Z. Biol. *63*, 411 (1914).

[3] P. N. R. USHERWOOD and H. GRUNDFEST, J. Neurophysiol. *28*, 497 (1965).

[4] I. PARNAS, B. C. ABBOTT, B. SHAPIRO and F. LANG, J. gen. Physiol. *50*, 2500 (1967).

[5] C. A. G. WIERSMA, in *The Physiology of Crustacea* (Ed. T. H. WATERMAN; Academic Press, New York 1961), vol. 2, p. 191.

[6] I. PARNAS and H. L. ATWOOD, Comp. Biochem. Physiol. *18*, 701 (1966).

[7] G. HOYLE and C. A. G. WIERSMA, J. Physiol. *143*, 402 (1958).

muscle, Dudel and Kuffler[8] estimated 50 excitatory synapses. The E-axon of the crayfish opener and stretcher muscles innervates about 1300 muscle fibres; thus the total number of synapses made by this axon is estimated at about 65,000[9] – a figure more comparable to a vertebrate central interneuron than to a vertebrate motor neuron. The common inhibitor of crabs may have an even larger number of synapses.

The different E- or I-axons of a given muscle usually have somewhat different properties. Those leg muscles receiving 2 E-axons can in many cases produce a fast or a slow contraction, depending upon which of the axons is stimulated; the mechanisms underlying this dual responsiveness have been discussed in a recent review[10]. The mechanisms include the presence of fast- and slow-contracting fibres within the muscle, which are differentially innervated by the 2 axons (Figure 2). In addition, the electrical responses evoked by the 'fast' axon are frequently larger than those of the 'slow' axon at the same frequency of stimulation[7,11], presumably due to the release of more transmitter substance from endings of the 'fast' axon. The 'fast' contractions are less affected by inhibitory stimulation than the 'slow' contractions[12]. This difference is related both to the larger average size of the 'fast' electrical responses, and to the fact that some fibres innervated by the fast axon have no inhibitory innervation, while all or most fibres innervated by the slow axon are also innervated by an I-axon (Figure 2).

The differences between the 2 I-axons of certain crustacean muscles have not been fully worked out. In crabs, the 'common inhibitor' is less effective in suppressing the contractions of the opener and stretcher muscles and in reducing the excitatory electrical events than the 'specific' inhibitors of these muscles[5,13]; but the reason for this remains to be determined.

Other general features of inhibitory axons (fatigue, facilitation etc.) are dealt with in an earlier review[5]. It is known that inhibition (measured as the degree of reduction of the muscle contraction set up by a brief stimulation of the E-axon) reaches maximum effectiveness after one to several seconds of stimulation of the I-axon, and that the inhibitory effect shows fatigue after $^1/_2$–2 min of steady stimulation (in the opener of the hermit crab *Eupagurus*). The rate of development of both facilitation and fatigue increases with the frequency of stimulation applied to the I-axon.

Inhibitory Transmitter Substance

It is believed that inhibition in crustacean muscles is mediated by the release of a chemical substance, the 'inhibitory transmitter', from the I-axon endings. The search for possible inhibitory transmitter substances has turned up γ-aminobutyric acid (GABA) as the most likely possibility. Originally GABA was shown to occur in high concentrations in the mammalian central nervous system and to block discharge of the crayfish stretch receptor[14]; subsequently it was found to have an inhibitory effect on crustacean muscle[15].

The claim for GABA as the inhibitory transmitter has been disputed[16], but the evidence in favour of the possibility seems at present much stronger than the opposing evidence.

The large size of crustacean neurons has made it possible to isolate individual axons and cell bodies for analysis by chemical, enzymatic and chromatographic methods. The I-axons[17] and the associated cell bodies[18] contain much more GABA than E-axons and their cell bodies. GABA content of an isolated I-axon has been estimated at about 0.5% of its wet weight, whereas in E-axons very little GABA can be detected. The difference in concentration is probably about 100 times. Glutamate, possibly the excitatory transmitter, is present in both axons.

The demonstration of GABA in I-axons is at variance with earlier results[16] which showed no GABA in these neurons. The reasons for the discrepancy are not clear.

More recently, GABA has been found in the perfusate collected from lobster muscle during stimulation of the I-axon[19]. The amount of GABA increased with

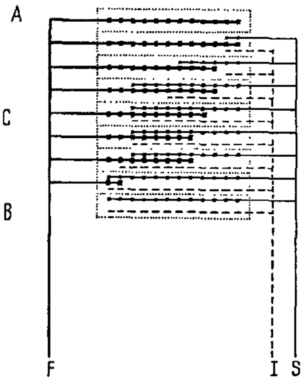

Fig. 2. Diagrammatic representation of the innervation of fibers within a crab closer muscle, showing distributions of fast (F), slow (S), and inhibitory (I) axons. Some fibers (A) receive only the fast axon; others (B) only the slow and inhibitory axons; but a majority (C) are triply innervated. (After Atwood[10].)

---

[8] J. Dudel and S. W. Kuffler, J. Physiol. *155*, 514 (1961).

[9] G. Bittner, Ph.D. Thesis, Stanford University (1967).

[10] H. L. Atwood, Am. Zool. *7*, 527 (1967).

[11] H. L. Atwood and G. Hoyle, J. Physiol. *181*, 225 (1965).

[12] C. A. G. Wiersma and C. H. Ellis, J. exp. Biol. *18*, 223 (1942).

[13] G. Hoyle and C. A. G. Wiersma, J. Physiol. *143*, 426 (1958).

[14] A. Bazemore, K. A. C. Elliot and E. Florey, J. Neurochem. *1*, 334 (1957).

[15] W. G. Van der Kloot and J. Robbins, Experientia *15*, 35 (1959).

[16] E. Florey and D. D. Chapman, Comp. Biochem. Physiol. *3*, 92 (1961).

[17] E. A. Kravitz, S. W. Kuffler and D. D. Potter, J. Neurophysiol. *26*, 739 (1963).

[18] M. Otsuka, E. A. Kravitz and D. D. Potter, J. Neurophysiol. *30*, 725 (1967).

[19] M. Otsuka, L. L. Iversen, Z. W. Hall and E. A. Kravitz, Proc. natn. Acad. Sci. USA *56*, 1110 (1966).

the duration and frequency of the stimulation; the average amount of GABA recovered per stimulus ranged from 1 to 4×10^{-14} moles. GABA was not recovered during stimulation of the E-axon, nor during I-axon stimulation when transmission was blocked presynaptically in low-Ca^{++} solution. Thus the site of release of GABA is the inhibitory nerve ending, and GABA is most likely the inhibitory transmitter.

The fate of GABA after normal release is not yet known, but it appears to be taken up by nerve or muscle[20]. In view of the restricted extracellular space at the inhibitory synapse, it is certainly conceivable that GABA inactivation after synaptic transmission is entirely due to the uptake mechanism, and not to activity of a synaptic enzyme concerned with GABA breakdown.

Since E- and I-axons both contain glutamate, and since GABA can be derived from glutamate by the action of glutamate decarboxylase (Figure 3), it is likely that one of the major differences between the 2 axons is the presence of the enzyme at higher levels in the I-axon. This difference has recently been directly demonstrated[21]. A further step in the analysis could involve study of the genetic control of the synthesis of this enzyme, to determine what regulatory factors govern its production.

Further evidence for GABA as the inhibitory transmitter is derived from its physiological effects, which mimic those seen during I-axon stimulation. The effects will be discussed in more detail below, but we may note here that they include the following:

(a) Both GABA application and inhibitory stimulation cause an increase in chloride ion conductance of the muscle fibre membrane[22].

(b) Both treatments reduce the amount of excitatory transmitter liberated by E-axon terminals in crayfish opener muscles[23].

(c) Neither of the above effects is evident in muscle fibres with sparse or no I-axon innervation ([24]; and see below).

Electrical Manifestations of Inhibition

Early studies on inhibition in crustacean muscle showed that in some muscles the excitatory potentials (excitatory postsynaptic potentials, or e.p.s.p.s) were reduced in amplitude (together with the tension output of the muscle) during stimulation of the inhibitory nerve, especially when an inhibitory nerve impulse was timed to arrive at the muscle 1–10 msec before each excitatory impulse. In other muscles, or with late arrival of the inhibitory impulse, the e.p.s.p.s were little reduced, yet the muscle contraction was effectively inhibited[25]. The 2 cases were designated 'supplemented inhibition' later, α-inhibition[26,27], and 'simple inhibition' (or β-inhibition) respectively. This terminology reflects the idea that the inhibitory actions on the e.p.s.p.s and the muscle contraction are somehow separate[5]. The original work was done with external electrodes, hence no information about inhibitory membrane polarization was obtained. The terminology does not specify the mechanisms by which the 2 types of inhibition are brought about, nor whether the same or different mechanisms are involved; it is purely phenomenological. In the light of more recent information about the mechanisms of inhibition, the original terminology has lost much of its usefulness, although it is still employed sometimes to describe effects for which the mechanism cannot be specified, e.g. in records of muscle potentials made with implanted wire electrodes[28].

The modern picture of the inhibitory effects specifies 2 mechanisms, one postsynaptic (on the muscle fibre membrane) and the other presynaptic (on the terminals of the E-axon). Both effects are mediated by a single I-axon (see Figure 10). The first mechanism can produce either α- or β-inhibition and the second always gives α-inhibition, as will appear in the following discussion. The first mechanism is found in more muscles than the second, and will be considered first.

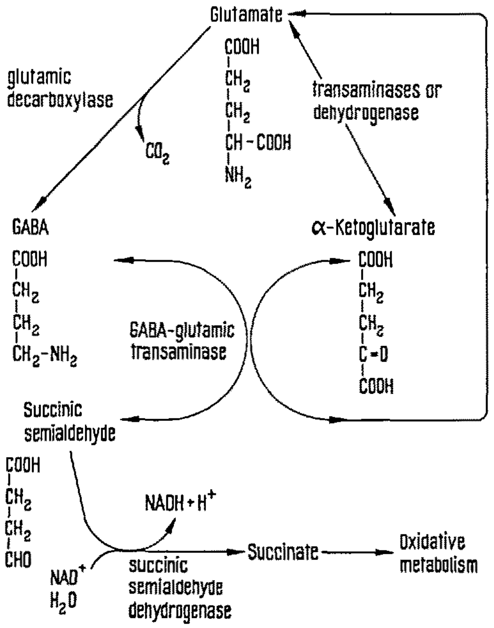

Fig. 3. Biochemical pathways in GABA metabolism. (After Kravitz et al.[17].)

---

[20] L. L. Iversen and E. A. Kravitz, Fedn. Proc. Fedn Am. Socs exp. Biol. *25*, 714 (1966).

[21] E. A. Kravitz, P. B. Molinoff and Z. W. Hall, Proc. natn. Acad. Sci. USA *54*, 778 (1965).

[22] J. Boistel and P. Fatt, J. Physiol. *144*, 176 (1958).

[23] J. Dudel and S. W. Kuffler, J. Physiol. *155*, 543 (1961).

[24] H. L. Atwood, Comp. Biochem. Physiol. *16*, 409 (1965).

[25] G. Marmont and C. A. G. Wiersma, J. Physiol. *121*, 318 (1938).

[26] S. W. Kuffler and B. Katz, J. Neurophysiol. *9*, 337 (1946).

[27] B. Katz, Biol. Rev. *24*, 1 (1949).

[28] H. L. Atwood and B. Walcott, Can. J. Zool. *43*, 657 (1965).

Postsynaptic Inhibition

Intracellular records from crustacean muscle fibres during I-axon stimulation often show inhibitory postsynaptic potentials (i.p.s.p.s), usually less than 5 mV, which can be either hyperpolarizing or depolarizing (Figure 4). Spontaneous miniature i.p.s.p.s occur in some crustacean muscle fibres, substantiating the quantal nature of this transmission[29]. In some fibres no i.p.s.p.s are recorded unless the membrane potential is displaced either above or below the resting level (e.g. by injection of current into the fibre with a second microelectrode). Systematic alterations of the muscle fibre membrane potential during I-axon stimulation reveals a reversal of polarity of the i.p.s.p. (from hyperpolarizing to depolarizing as the membrane potential is increased). The 'reversal potential' is usually very close to the resting potential[13,22,30].

As in other well-studied cases of postsynaptic inhibition[31], the i.p.s.p. in crustacean muscle results from an increase in membrane conductance for an ion with an equilibrium potential close to the resting membrane potential. In this case, the ion involved is chloride[22,32,33]. Replacement of chloride in the extracellular solution with a large anion (such as pyroglutamate) shifts the reversal potential for the i.p.s.p. and for GABA action in crayfish muscle closer to zero level, in accordance with the predicted shift of the chloride ion equilibrium potential, E_{Cl^-} ($E_{Cl^-} \simeq 58$ mV. log[internal Cl^-]/[external Cl^-]). TAKEUCHI and TAKEUCHI[33] have estimated that the contribution of K^+ to the i.p.s.p. is less than 10%, unlike the case in certain vertebrate neurons[31]. Recently MOTOKIZAWA et al.[32] have demonstrated that the lobster I-axons effect a permeability increase to certain other anions, including acetate and propionate, although the permability for these anions is very low compared with that for chloride. Thus the inhibitor-controlled ionic channels are not completely specific for chloride ion.

The inhibitory postsynaptic receptors have been studied by application of GABA, either iontophoretically[34] or in the solution surrounding the muscle fibre[33,35]. In the crayfish opener muscle the GABA receptors are located very close to glutamate receptors, which are activated by iontophoretically applied glutamate, generating membrane depolarization[36]. The glutamate receptors are thought to be identical with those reactive to the E-axon transmitter, and the GABA receptors likely represent those for the I-axon transmitter. A double-barrelled microelectrode filled with GABA and glutamate (in separate chambers) can stimulate both types of receptor when placed at a single point on the muscle fibre. Thus the 2 types of synapse are thought to occur together, and this is borne out in electron micrographs (see below). The 2 types of receptor are kinetically different. Those for glutamate undergo rapid desensitization, whereas those for GABA remain activated as long as this substance is present in sufficient concentration.

The threshold concentration of GABA for the inhibitory conductance change in muscle fibres of the crayfish opener muscle is about $10^{-6} M$; full activation occurs at about $5 \times 10^{-4} M$[33]. The size of the conductance change varies with the pH of the solution, being smaller at low pH. From these data, TAKEUCHI and TAKEUCHI[33] postulate that there is a fixed positive charge associated with the receptors (or with the membrane channels controlled by the receptors), which is sensitive to pH.

The inhibitory receptors are inactivated by picrotoxin, and are activated by a number of other compounds besides GABA, although the latter compound is apparently the most effective in this respect[35].

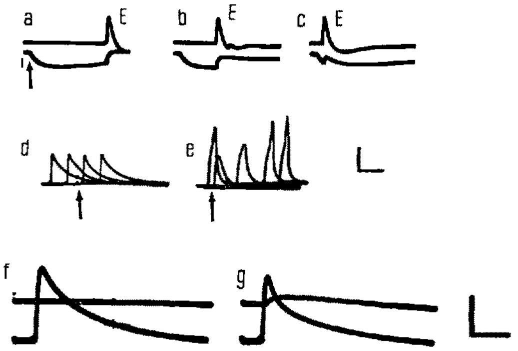

Fig. 4. Postsynaptic inhibitory effects in the closer muscle of *Pachygrapsus* (a–c) and in deep extensor muscles (d–g); for innervation, see Figure 1). Inhibitory postsynaptic potentials are evident in a–c and in g; arrows show the beginning of the i.p.s.p.s. in a, d, and e. In a–c, e.p.s.p.s. (E) were recorded simultaneously in type A (top) and type C (bottom) muscle fibers of Figure 2, at various times after the i.p.s.p.; note reduction of the lower e.p.s.p. with close timing, and lack of effect on the upper e.p.s.p. In d and e, e.p.s.p.s. (d) and e.p.s.p.s. with an electrically excited membrane response (e) were timed to arrive at various times relative to the i.p.s.p. The electrically excited component was eliminated with close timing (e), but the 'pure' e.p.s.p. was little reduced (d), indicating little or no presynaptic inhibition. In f and g, e.p.s.p.s. (bottom) were recorded in L_2 in response to axon 4 (see Figure 1), while another electrode was inserted in M to record the i.p.s.p. of axon 5 (g, top). The increased decay rate and slightly reduced amplitude of the e.p.s.p. during inhibitory activity, are clearly evident. Voltage scale: 20 mV (a–c), 10 mV (f, g); time scale, 40 msec (a–c), 20 msec (d–g). (After ATWOOD[10] and ATWOOD et al.[38].)

---

[29] H. GRUNDFEST and J. P. REUBEN, in *Nervous Inhibition* (Ed. E. FLOREY; Pergamon Press, Oxford 1961), p. 92.

[30] P. FATT and B. KATZ, J. Physiol. *121*, 374 (1953).

[31] J. C. ECCLES, *The Physiology of Synapses* (Academic Press, New York 1964).

[32] F. MOTOKIZAWA, J. P. REUBEN and H. GRUNDFEST, J. gen. Physiol. *50*, 2491 (1967).

[33] A. TAKEUCHI and N. TAKEUCHI, J. Physiol. *191*, 575 (1967).

[34] A. TAKEUCHI and N. TAKEUCHI, J. Physiol. *177*, 225 (1965).

[35] H. GRUNDFEST, J. P. REUBEN and W. H. RICKLES, J. gen. Physiol. *42*, 1301 (1959).

[36] A. TAKEUCHI and N. TAKEUCHI, J. Physiol. *170*, 296 (1964).

It is interesting to note that not all crustacean muscle fibres show a membrane conductance change when treated with GABA. Particularly in crab leg muscles, GABA-insensitive fibres occur[24,37]. They show little or no anatomical or physiological evidence of inhibitory innervation[38]. No i.p.s.p.s appear, and there is no demonstrable membrane conductance change during stimulation of the inhibitory axon. Apparently, GABA receptors appear on crustacean muscle fibres only in conjunction with inhibitory innervation.

The effect of postsynaptic inhibition is to maintain the muscle fibre membrane potential at the chloride equilibrium potential, or to drive it towards this level if displaced by excitatory postsynaptic activity. Thus some reduction of the e.p.s.p. is usually evident when an i.p.s.p. occurs simultaneously (Figure 4), although in many cases the reduction is not more than 5%. In addition, the membrane potential may show hyperpolarization when i.p.s.p.s are closely spaced in time, and the e.p.s.p.s usually show acceleration of decay rate (Figure 4). All of these effects result in less overall depolarization of the muscle fibre for a given excitatory input. Even though the reduction in amplitude of individual e.p.s.p.s may be small, the muscle fibre will develop less tension, because the average membrane potential is held more polarized. These considerations are thought to be adequate to explain the phenomenon of β-inhibition, referred to above.

Some crustacean muscle fibres develop large spike-shaped membrane responses during E-axon activity. These can be very effectively cancelled by the I-axon (Figure 4). The increased movement of chloride ion across the membrane during the i.p.s.p. is apparently sufficient to nullify or prevent the large inward flow of positive ion responsible for the active membrane response[38].

An early hypothesis of inhibitory action in crustacean muscle based the inhibition of contraction on a direct effect on the contractile substrate, without mediation of the membrane potential[5]. All recent experiments have failed to support this view. Tension records from single innervated muscle fibres have shown that tension is completely abolished during inhibitory action only when the membrane potential becomes more polarized than the level at which tension is normally initiated by depolarization[39]. At membrane potentials more depolarized than this level, I-axon activity reduces muscle fibre tension only to the extent expected from the associated change in membrane potential. In single crayfish muscle fibres exposed to solutions containing GABA, the membrane potential-tension relationship and the threshold membrane potential for tension development are unchanged[40]. These results support the view that the I-axons influence muscle tension by regulation of the muscle fibre membrane potential, either directly (postsynaptic inhibition) or indirectly (presynaptic inhibition).

Presynaptic Inhibition

MARMONT and WIERSMA[25], KUFFLER and KATZ[26] and FATT and KATZ[30] observed that the extent of inhibitory reduction of an e.p.s.p. depended on the time of arrival of the I-axon impulse. The largest reductions occurred when the inhibitory impulse arrived at the muscle 1–10 msec before the E-axon impulse. The large reduction could not be accounted for on the basis of the observed decrease in muscle fibre membrane resistance brought about by inhibition. In some fibres, the extent of the latter effect would predict a 5% reduction of the e.p.s.p. whereas reduction of 80–90% is observed[30]. KUFFLER and KATZ[26] and FATT and KATZ[30] proposed a curare-like competitive inhibition of the E-axon postsynaptic receptors by the I-axon transmitter. The latter authors state: 'The attenuation of the end-plate potential by an inhibitory nerve impulse could be explained by a competition between 2 antagonistic transmitter substances, reacting with a single receptor substance.' This hypothesis predicts a reduction in size of the quantal unit of the e.p.s.p., due to reduction in postsynaptic effectiveness of released transmitter[41].

DUDEL and KUFFLER[23] were able to record from single E-axon synaptic regions of the crayfish opener muscle by means of an extracellular microelectrode placed in the solution just over the synapse. Such an electrode measures a voltage drop in the solution during flow of current across the postsynaptic membrane. The voltage drop (externally recorded synaptic potential, or e.r.s.p.) provides a measure of quantal size and also quantal content of the e.p.s.p. Amplitude histograms of the e.r.s.p.s measured during stimulation of the E-axon showed several peaks centred at multiples of the quantal unit, which was equal in size to spontaneously occurring e.r.s.p.s (Table I). The amplitude histograms were accurately described by Poisson's theorem, which has been applied successfully by DEL CASTILLO and KATZ[42] to the end-plate potentials of Mg^{++}-treated frog muscles.

When I-axon impulses were timed to arrive 1–4 msec before the motor impulses, the quantal content of the e.r.s.p.s was reduced, but quantum size remained the same. This effect was seen in the amplitude histograms, which showed a larger proportion of events in the 0-quantum (failure) and 1-quantum categories (as compared with the 2- and 3-quantum groups) but no

---

[37] E. FLOREY and G. HOYLE, in *Nervous Inhibition* (Ed. E. FLOREY; Pergamon Press, Oxford 1961), p. 105.

[38] H. L. ATWOOD, I. PARNAS and C. A. G. WIERSMA, Comp. Biochem. Physiol. *20*, 163 (1967).

[39] H. L. ATWOOD, G. HOYLE and T. SMYTH, J. Physiol. *180*, 449 (1965).

[40] R. K. ORKAND, J. Physiol. *164*, 103 (1962).

[41] B. KATZ, Proc. R. Soc. B *155*, 455 (1962).

[42] J. DEL CASTILLO and B. KATZ, J. Physiol. *124*, 560 (1954).

change in mean amplitudes of the 1-quantum peak or the spontaneous potentials (Table I). The experiment rules out the competitive inhibition mechanism of FATT and KATZ[30] and indicates that instead, the output of excitatory transmitter is reduced by appropriately timed I-axon impulses. The effect could arise from an action of the inhibitory transmitter on the E-axon terminals, i.e. presynaptic inhibition.

Subsequent work has supported the suggestions of DUDEL and KUFFLER[23] and DUDEL[43], namely, that the presynaptic effect is brought about by an action of the inhibitory transmitter on the E-axon terminal which increases the chloride ion conductance of the terminal membrane, in a manner resembling the postsynaptic effect on the muscle fibre membrane. The conductance increase is thought to reduce the amplitude of the nerve terminal potential (n.t.p.) of the E-axon, and thus to decrease the output of the excitatory transmitter, on the assumption that the release process is controlled by the amplitude of voltage changes in the nerve terminal[44].

The reduction of the externally recorded n.t.p. (Figure 5) has been analyzed in some detail in the crayfish opener muscle by DUDEL[45,46], who used a computer of average transients to study the rather small potentials.

Depending on its location, an extracellular electrode records 2 size classes of nerve terminal potentials at the surface of muscle fibres in the opener. The larger potentials are triphasic or biphasic (termed class I potentials below) while the smaller potentials are biphasic or monophasic (termed class II potentials below; see Figure 6). DUDEL suggests that class I potentials are recorded when the electrode is located some distance from the synapse while class II potentials are recorded when the electrode is at or near the ultimate nerve terminal. While the evidence in support of this conclusion is not as complete as one might like, it is highly suggestive. First, class I potentials are found rather easily and are often recorded first when a synaptic area is being explored[47]. This might be predicted if, as believed, the ultimate nerve terminal represents a rather small area. Secondly, when the electrode is shifted closer to the ultimate nerve terminal (as indicated by an increase in size of the e.r.s.p.s.) the amplitude of the nerve terminal potentials decreases; at the location of the maximum e.r.s.p. a 'pure' class II potential (monophasic and of minimum size) is recorded. Since conduction at and near the ultimate nerve terminal is believed to be electrotonic, due to

Table I. Effects of inhibitory impulses timed to arrive just before excitatory impulses on excitatory transmitter release (data from DUDEL and KUFFLER[23])

	Excitor (1)	Stimulation (5/sec) Excitor + inhibitor (2)
Impulses releasing 0 quanta (failures)	7%	57%
Impulses releasing 1 quantum	28%	28%
Impulses releasing 2 quanta	30%	13%
Impulses releasing > 2 quanta	35%	2%
Quantum content/ nerve impulse	2.4	0.56
Quantum size (± SD)	41 mV (± 11)	40 mV (± 10)

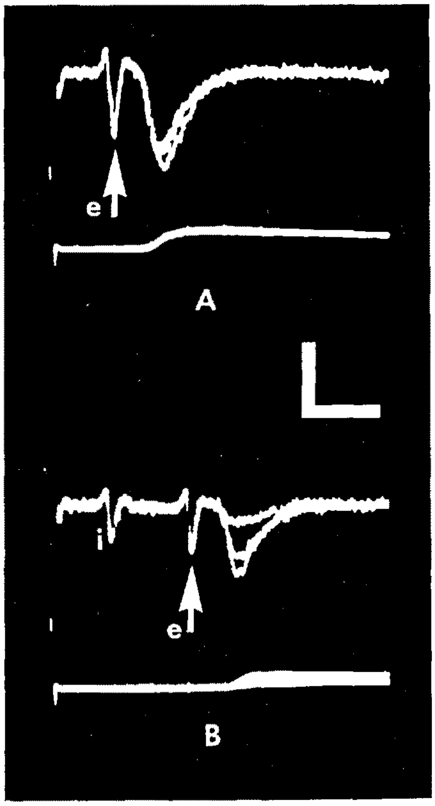

Fig. 5. Presynaptic inhibitory effects in the stretcher muscle of *Pachygrapsus*. External recordings of synaptic currents and nerve terminal potentials appear in the upper traces, and internally recorded e.p.s.p.s. in the lower traces. Excitatory n.t.p.s. (e) are marked by arrows; an inhibitory n.t.p. (i) appears in the upper trace in B. (A) Responses to 3 successive stimuli to the E-axon at 1/sec. (B) Arrival of inhibitory impulses reduces the excitatory n.t.p., the synaptic current, and the e.p.s.p. Calibration: voltage, 400 μV (top traces), 20 mV (bottom traces); time, 4 msec. (After ATWOOD and JONES[59].)

---

[43] J. DUDEL, Nature *193*, 587 (1962).

[44] K. KUSANO, D. R. LIVENGOOD and R. WERMAN, Science *155*, 1257 (1967).

[45] J. DUDEL, Pflügers Arch. ges. Physiol. *277*, 537 (1963).

[46] J. DUDEL, Pflügers Arch. ges. Physiol. *284*, 66 (1965).

[47] J. DUDEL, Pflügers Arch. ges. Physiol. *282*, 323 (1965).

incomplete invasion of the spike, this observation follows logically. Triphasic and biphasic potentials would be expected at and near regions of active spiking, while biphasic and monophasic potentials of decreasing amplitude would be expected as the electrode is moved farther away from the region of active spikes into the electrotonically conducting region of the nerve terminal (Figure 6).

Dudel[45,46] found that class II potentials invariably show reduction during I-axon stimulation (Figure 5). Class I potentials, on the other hand, sometimes show an increase in amplitude during presynaptic inhibition. Dudel argues that these effects would follow if the nerve terminals conduct electrotonically and undergo an increase in chloride conductance (and consequent decrease in membrane resistance) during presynaptic inhibition, and provided the class I potentials are recorded near a spike-generating locus of the axon. The éffect is illustrated in Figure 6. During the inhibitory conductance increase the electrotonic current flowing into the non-conducting nerve terminal from the current source (which is the spiking locus of the axon) will remain constant, but a larger fraction will pass across the nerve terminal membrane at points relatively close to the current source. Thus during inhibition the class I n.t.p.s which are recorded near the current source, where transmembrane current flow has increased, will appear larger, while class II potentials recorded at some distance from the current source, where transmembrane current has decreased, will appear smaller.

These observations seem to rule out marked hyperpolarization or depolarization of the nerve terminals as an inhibitory mechanism, in contrast with presynaptic inhibition in certain places in the vertebrate central nervous system[48]. Neither of these alternatives can explain the differential change in size of the n.t.p. at different loci during presynaptic inhibition, whereas the membrane resistance change postulated by Dudel[45,46] accounts for it very nicely.

GABA and related drugs such as guanidino-acetic acid duplicate the action of the I-axon on the excitatory nerve terminals: they reduce the output of transmitter, and reduce the n.t.p.[23,49,50]. β-guanidino-propionic acid and related drugs also reduce the output of excitatory transmitter without altering quantal size, but this group of drugs does not effect an increase in chloride ion conductance in the muscle fibre membrane[49]. Thus there is evidence that receptors at the 2 sites differ, those of muscle being more specific for GABA-type compounds.

Further evidence for an increase in chloride ion permeability of the excitatory terminal membrane as the basis for presynaptic inhibition comes from the work of Takeuchi and Takeuchi[34,51] who found that the n.t.p. is not significantly reduced by I-axon or GABA action when the chloride of the extracellular solution is replaced by propionate, or by other large, presumably poorly penetrating anions.

In fibres of crab muscles thought to receive no inhibitory innervation, the e.r.s.p.s and n.t.p.s show no significant reduction in solutions with raised GABA concentration (10^{-4} g/ml). In other crab muscle fibres which do receive I-axon innervation, the inhibitory effects of GABA or the I-axon are pronounced[52]. These comparative observations provide further evidence that GABA sensitivity of nerve terminals is associated with the presence of the I-axon, and that a GABA-mediated change in the nerve terminal is a likely basis for presynaptic inhibition.

Distribution of Pre- and Postsynaptic Inhibition

Although inhibitory innervation of crustacean muscles is almost universal, not all fibres within a muscle show inhibitory effects to the same degree[24,38,53]. Some fibres, particularly in crabs, probably receive

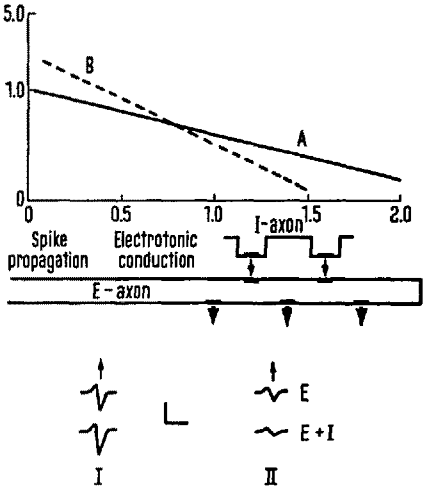

Fig. 6. Diagram to illustrate Dudel's[45,46] explanation for the presynaptic inhibitory effects on the crayfish excitatory n.t.p. Representative recordings of class I and class II potentials (see text) are illustrated with (E + I) and without (E) presynaptic inhibition; the regions of the axon from which they are believed to be recorded are also indicated. The exact location and number of axo-axonal synapses are not known. Postulated change in terminal resistance may alter the length constant of the nerve terminal and give rise to an altered distribution of electrotonic membrane current, as indicated in the top graph, where A and B represent possible conditions before and during presynaptic inhibition, respectively. In the graph, the abscissa represents the distance along the terminal divided by the length constant of the terminal, and the ordinate represents the electrotonic membrane current. For the electrical recordings, the voltage scale is 20 μV and the time scale, 2 msec.

---

[48] J. C. Eccles, R. F. Schmidt and W. D. Willis, J. Neurophysiol. *26*, 523 (1963).

[49] J. Dudel, Pflügers Arch. ges. Physiol. *283*, 104 (1965).

[50] A. Takeuchi and N. Takeuchi, J. Physiol. *183*, 418 (1966).

[51] A. Takeuchi and N. Takeuchi, J. Physiol. *183*, 433 (1966).

[52] H. L. Atwood, unpublished observations.

[53] R. Werman, J. P. Rueben and H. Grundfest, Biol. Bull. *119*, 347 (1960).

no inhibitory innervation (Figure 2). In addition, some crustacean muscles do not show the presynaptic component of inhibition; the postsynaptic mechanism is apparently more general.

In the slow abdominal flexor muscles of the crayfish, postsynaptic inhibition is present in some fibres, as shown by inhibitory reduction in amplitude of the e.p.s.p.s and of membrane voltage responses to pulses of injected current. However, no matter what temporal relationship the I-axon impulse has to the e.p.s.p., the latter is reduced no more than an artificially applied voltage pulse of similar amplitude and timing. Thus the whole of the inhibitory reduction of the e.p.s.p. is attributable to the change in muscle membrane resistance accompanying the i.p.s.p.[54]. In the abdominal extensor muscles of the crayfish, also, the small amount of reduction of the e.p.s.p.s is most likely attributable to postsynaptic action[38].

Presynaptic inhibition has been described so far only in the walking leg and claw opener muscles of crayfish and lobster, and in the opener and stretcher muscles of crab (*Pachygrapsus*) walking legs[10,23,54]. Undoubtedly the phenomenon is widespread in other crustacean leg muscles, as indicated by data in the earlier literature[5,30]. Within the muscles endowed with the presynaptic mechanism, the amount of reduction of the e.p.s.p. which can be effected by this mechanism may be variable. Bittner[55] has observed that the distal fibres in the claw opener muscle of the crayfish *Procambarus clarki*, which have poorly facilitating e.p.s.p.s[9], are more likely to show a large reduction of the e.p.s.p. through presynaptic inhibition than the central fibres, which have strongly facilitating e.p.s.p.s. These results suggest a rather complex synaptic organization, which invites more complete exploration.

One question arising from the above work which has not been satisfactorily dealt with concerns the relative effectiveness of the 2 types of inhibition in controlling muscle tension. Wiersma and co-workers[5,13] observed little difference in effectiveness between α- and β-inhibition in preparations showing both phenomena. By contrast Kuffler and Katz[26] found that inhibition of contraction in the opener of an Australian crayfish species was more rapid and complete during α-inhibition. If the tension is controlled by muscle fibre membrane potential, the latter effect would be the one expected, since the reduced p.s.p.s would yield a smaller degree of membrane depolarization. Thus there is evidence that the presynaptic mechanism contributes in some cases at least to the effectiveness of inhibition. However, the problem deserves further investigation, in view of conflicting evidence.

Electron Microscopy of Crustacean Neuromuscular Synapses

In an effort to elucidate the anatomical basis for the pre- and postsynaptic inhibitory effects, electron microscopic observations have been made with several different crustacean muscles, including the closer muscle of *Pachygrapsus* (in which there are fibres with no inhibitory input), the slow abdominal muscles of the crayfish (in which there are fibres with sparse or no inhibitory innervation), and the opener muscle of the crayfish (in which pre- and postsynaptic inhibition are both well developed).

Muscle fibres selected for lack of inhibitory innervation[10,56,57] have been found to possess nerve endings with predominantly round synaptic vesicles (Figure 7). The nerve endings are usually covered by the sarcolemma or embedded in clefts invaginating from the surface of the muscle fibre. Synaptic contacts were identified on the basis of electron-dense pre- and postsynaptic membranes and an aggregation of presynaptic vesicles at the darkened presynaptic membrane of the nerve ending. The synaptic gap is usually of the order

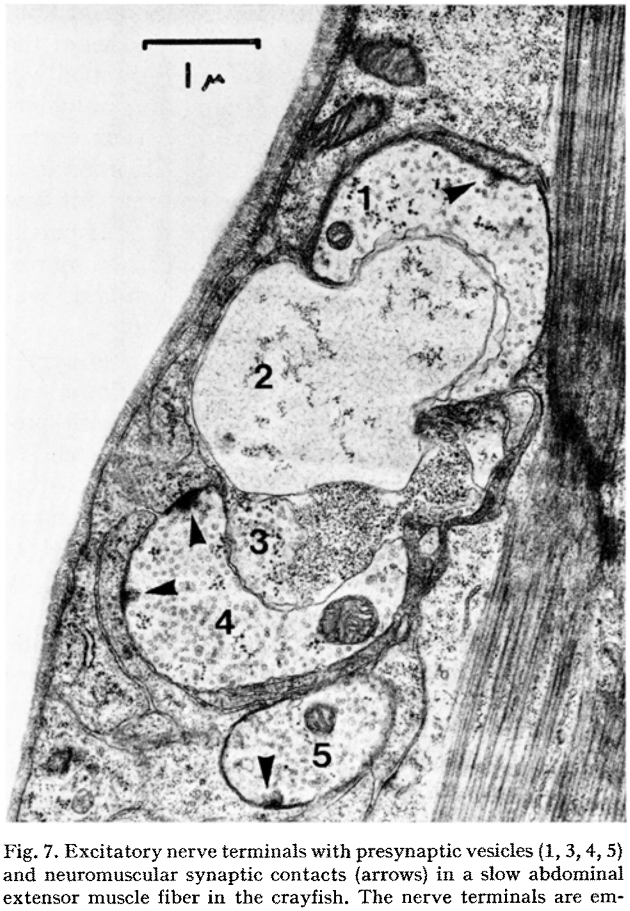

Fig. 7. Excitatory nerve terminals with presynaptic vesicles (1, 3, 4, 5) and neuromuscular synaptic contacts (arrows) in a slow abdominal extensor muscle fiber in the crayfish. The nerve terminals are embedded in arms of the muscle fiber near the surface; contractile filaments appear at right. The presynaptic vesicles are all round. (After Jahromi and Atwood[56].)

[54] D. Kennedy and W. H. Evoy, J. gen. Physiol. *49*, 457 (1966).

[55] G. Bittner, unpublished observations.

[56] S. S. Jahromi and H. L. Atwood, Can. J. Zool. *45*, 601 (1967).

[57] H. L. Atwood and H. S. Johnson, J. exp. Zool., in press.

of 200 Å, which is considerably less than the 500 Å gap of frog neuromuscular synapses, and resembles more the situation in synapses of the central nervous system[31]. Characteristically, in the electron micrographs, 2 or more synaptic contacts occur close together on the same nerve ending, and synapses of adjacent nerve endings are often close together (Figure 7).

In muscles possessing both excitatory and inhibitory innervation, 2 types of endings have been found[58,59]. In addition to the first type with round presynaptic vesicles, a second type with vesicles of more irregular shape occurs, often in close proximity to endings of the first type (Figure 8). The closeness of the 2 endings and their presence near the surface of the muscle fibre suggest that the differences between them are not produced by variable or poor fixation. Statistical comparisons have shown that the vesicles in the second type of ending are significantly smaller (in width), and significantly more elongated, than those in the first type of ending (Table II).

The differences between the 2 types of ending, and their locations, strongly indicate that the first is excitatory (E-type) and the second, inhibitory (I-type). The I-type endings form synaptic contacts with the muscle fibres, similar to those of the E-type.

The existence of the axo-axonal synapses reinforces the conclusions about the identity of the 2 types of ending. In the crayfish opener muscle, cases have been found in which I-type endings make synaptic contact with the E-type endings[59]. As in the neuromuscular synapses, electron-dense pre- and postsynaptic membranes are separated by a gap of about 200 Å. Vesicles appear accumulated on the I-axon side of the synapse, but not on the E-axon side (Figures 8 and 9). By analogy with other chemically transmitting synapses, this configuration suggests that transmission at this synapse occurs from the I-axon to the E-axon, as required for presynaptic inhibition. Probably these synapses mediate the presynaptic effect; the delay of about 1 msec associated with the appearance of presynaptic inhibition is about right for a chemical synapse, and too long for direct electrical transmission[23].

Table II. Characteristics of presynaptic vesicles in electron micrographs of crustacean nerve endings[57,59]

Ending	Mean maximum diameter (Å°)	Mean minimum diameter (Å°)	Maximum/ minimum
Pachygrapsus (E type)	540	447	1.21
Crayfish opener (E type)	535	445	1.20
Crayfish opener (I type)	507	330	1.54

In the opener muscle of the crayfish (*Orconectes virilis*) the axo-axonal synapses are more efficient in reducing the e.p.s.p. than the inhibitory neuromuscular synapses. Although the axo-axonal synapses are seen much less frequently in the electron micrographs, most of the observed reduction of the e.p.s.p. at frequencies

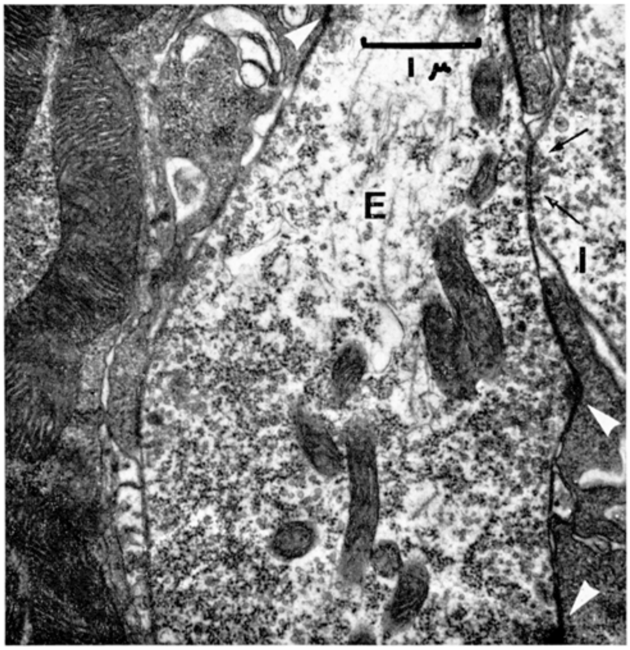

Fig. 8. Electron micrograph of excitatory (E) and inhibitory (I) terminals in the crayfish opener, showing axo-axonal synapse (black arrows) and excitatory neuromuscular synapses (white arrows).

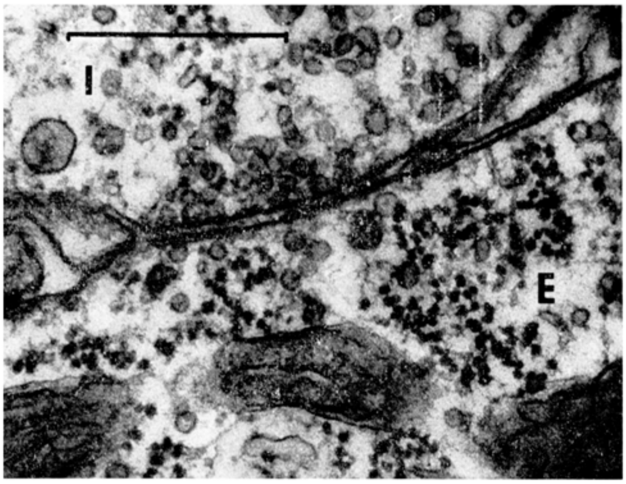

Fig. 9. Enlargement of an axo-axonal synapse, showing accumulation of vesicles in the I-axon at the (presynaptic) electron-dense membrane, and the synaptic gap. Scale mark, 0.5 μm. (After Atwood and Jones[59].)

---

[58] K. Uchizono, Nature *214*, 833 (1967).

[59] H. L. Atwood and A. Jones, Experientia *23*, 1036 (1967).

of stimulation of the I-axon below 20/sec is attributable to them. Postsynaptic reduction of e.p.s.p. amplitude appears only at higher frequencies of stimulation of the I-axon[60]; however, tension reduction due to postsynaptic inhibition undoubtedly appears at the lower frequencies[26]. This tension reduction is probably attributable to polarization of the muscle fibre membrane and to shortening of the e.p.s.p.s, as discussed previously.

The greater efficiency of the axo-axonal synapses compared with the inhibitory neuromuscular synapses is apparently in part a consequence of the much smaller membrane area of the excitatory nerve terminal in comparison with that of the muscle fibre. The change in membrane resistance due to activity of a single synapse would be much greater in the nerve terminal on this consideration.

The general scheme for the arrangement of I-axon synapses is shown diagrammatically in Figure 10.

Functional Significance of Peripheral Inhibition

Several explanations regarding the use to which the I-axons are put in normal activity have appeared in the literature. In the first place, it is clear that they participate in certain reflex patterns. BUSH[61,62] has studied reflex activation of the I-axons of crustacean leg muscles. Stroking the inside surface of the claw elicits a strong closing reaction, coupled with an inhibitory discharge to the antagonistic opener muscle, which serves to abolish any tension which would oppose the closing response. Also, passive stretch of a leg joint serves to activate a 'resistance reflex' in the muscle opposing the applied stretch, together with an inhibitory discharge to the antagonist of the reflexly activated muscle.

For the crayfish claw opener, the ratio of inhibitory to excitatory impulses determines the tension in the muscle. A ratio of about 3:4 is sufficient to give just complete inhibition. A lower ratio of inhibitory impulses permits muscle contraction to occur. During most normal activities, both axons are active, but the ratio of impulses in the 2 fluctuates[63]. Stimulation applied to the body of the animal, which presumably activates the motor and inhibitory neurons via a pathway involving tactile receptors and 'command' interneurons, causes an increased discharged in both E- and I-axons, but the E-axon discharge is dominant, with the result that the opener muscle contracts, and the claw opens. However, when the stretch receptor attached to the dactylopodite is activated (as by opening the claw), the ratio of inhibitory impulses is increased, although the E-axons may also show some increased activity. The increased inhibitory action serves as a negative feedback tending to stabilize the position of the claw (Figure 11).

Records of muscle potentials from legs of walking crabs suggest that the I-axons are highly active during the initial phase of a burst of E-axon activity. This would result in the uncoupling of tension production from excitatory neuromuscular facilitation, in a manner analogous to the operation of a clutch in an automobile motor[28]. It is proposed that this action would permit a more rapid development of tension upon release of the inhibitory 'clutch' (i.e. cessation of I-axon activity).

Another suggestion regarding the function of inhibition is that it may cause relaxation of tension in certain muscle fibres which normally remain in weak contracture[64].

None of the above explanations applies to the superficial flexor muscles of the crayfish abdomen. The I-axons of these muscles are normally active only when discharges in the E-axons have been inhibited centrally (for example, during abdomen extension)[65]. Conversely, the I-axon is silent when the E-axons are active. Activity in the I-axon has no appreciable effect

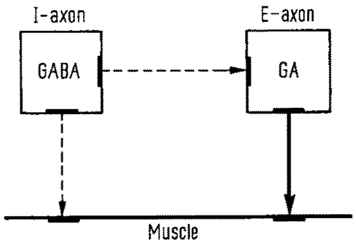

Fig. 10. Diagrammatic illustration of synaptic contacts in the crayfish opener muscle. Inhibitory transmission, dotted arrows; excitatory transmission, solid arrows; GABA, γ-aminobutyric acid; GA, glutamic acid.

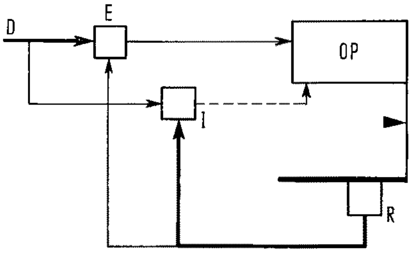

Fig. 11. Function of inhibition in the crayfish opener muscle, based on the account of WILSON and DAVIS[63]. Inhibition, dotted arrow; excitation, solid arrows; strong excitatory input or feedback, thick solid arrows. Opener muscle, OP; stretch receptor of the dactyl, R; excitatory neuron, E; inhibitory neuron, I; 'motor command' or 'driver' interneuron, D. Contraction of OP activates R, which leads to inhibition of the opener muscle.

---

[60] W. A. MORIN and H. L. ATWOOD, unpublished observations.
[61] B. H. BUSH, J. exp. Biol. *39*, 71 (1962).
[62] B. H. BUSH, Comp. Biochem. Physiol. *10*, 273 (1963).
[63] D. M. WILSON and W. J. DAVIS, J. exp. Biol. *43*, 193 (1965).
[64] P. N. R. USHERWOOD, Am. Zool. 7, 553 (1967).
[65] D. KENNEDY and K. TAKEDA, J. exp. Biol. *43*, 229 (1965).

on the rate of relaxation of tension following cessation of E-axon activity, nor upon resting muscle tension[66]. Since the I-axon innervates mostly those muscle fibres receiving several E-axons[65], the suggestion can be made that it acts as a 'safety' or 'anti-noise' device for eliminating accidental tension ('noise') in those muscle fibres statistically most likely to experience it (due to escape from central inhibition of E-axons). This feature may appear redundant, but is less so than many other nervous control systems.

The role of the common inhibitory axon of crab legs, which innervates antagonistic muscles, is also obscure. WIERSMA[67] has suggested that it is active only during molting, when overall inhibition of the muscles is required. It is of interest to note that the 'slow' motor systems of the doubly-motor-innervated muscles are much more influenced by this axon than the antagonistic, singly-motor-innervated muscles[12]. The axon could therefore act as a differential inhibitor of the doubly-motor-innervated muscles[28]; but such interpretations must await further work on the normal activity of the axon.

The inhibitory axons apparently represent a device for shifting some of the burden of regulation of muscle contraction from the central nervous system (which is not richly endowed with neurons compared to that of most vertebrates) to the muscle itself. Since muscle fibres conducting large spikes are not the rule in crustacean muscle, full use can be made of the integrative properties of the muscle fibre membrane and the entire range of tension production of each muscle fibre exploited.

Peripheral Inhibition in Other Arthropods

There is now substantial evidence for the presence of I-axons remarkably similar to those of crustaceans, in certain insect muscles[3,54]. Some controversy remains concerning the inhibitory function of these axons[68], but the pharmacological and electrical similarities between the insect and crustacean axons are striking, and there is evidence that the insect axons subserve an inhibitory role in the normal animal[64]. So far only postsynaptic inhibition is known. The insect I-axons (like that of the *Pachygrapsus* closer[38]) are usually distributed to a rather small proportion of the total fibres in a muscle. Only fibres receiving a 'slow' axon have inhibitory innervation in locust and grasshopper leg muscles[3].

Recently, inhibitory axons have also been reported in *Limulus* muscle[4]. So far they have not been found in giant barnacle[69] or in spider[70] muscles.

Other arthropod groups have not been critically examined[71].

Zusammenfassung. Krustazeenmuskeln sind von Axonen innerviert, die kontraktionshemmend sind. Die Hemmung wird durch Freisetzung einer chemischen Überträgersubstanz an den Nervenendigungen hervorgerufen. Beim Überträgerstoff handelt es sich wahrscheinlich um γ-Amino-Buttersäure (GABA), die in hoher Konzentration in den hemmenden Axonen vorhanden ist und während der Nervenerregung freigesetzt wird. GABA verdoppelt die physiologischen Effekte des Hemmungsvorganges. Diese erzielten Effekte können in 2 Kategorien eingeteilt werden: (1) Postsynaptische Hemmungsreaktion des hemmenden Überträgerstoffs mit Muskelfasermembran. (2) Präsynaptische Hemmungsreaktion mit erregenden Nervenendigungen. Die Grundlage für beide Effekte ist eine Erhöhung der Chlorionen-Leitfähigkeit der reagierenden Membranen. Der Nachweis postsynaptischer Hemmung schliesst das Auftreten hemmender Postsynapsenpotentiale und eine erhöhte Abfallrate des erregenden postsynaptischen Potentials mit ein. Während einer präsynaptischen Hemmung ist das Potential der erregenden Nervenendigungen verkleinert und die Freisetzung des erregenden Überträgerstoffs verringert. Studien am Elektronenmikroskop haben hemmende synaptische Kontakte mit den Muskelfasern und den erregenden Nervenendigungen gezeigt. Die normale Funktion hemmender Axone konnte nur in bestimmten Muskeln erklärt werden.

---

[66] W. H. EVOY, D. KENNEDY and D. M. WILSON, J. exp. Biol. 46, 393 (1967).

[67] C. A. G. WIERSMA, J. comp. Neurol. *74*, 63 (1941).

[68] G. HOYLE, J. exp. Biol. *44*, 413 (1966).

[69] G. HOYLE and T. SMYTH, Comp. Biochem. Physiol. *10*, 291 (1963).

[70] W. RATHMAYER, Comp. Biochem. Physiol. *14*, 673 (1965).

[71] Some of the observations described in this account were obtained by the author in studies supported by The National Research Council of Canada and the Muscular Dystrophy Association of Canada. The author is indebted to Mr. G. WHEATLEY for a critical reading of the manuscript.