Presynaptic Inhibition at Inhibitory Nerve Terminals. A New Synapse in the Crayfish Stretch Receptor

(Procambarus/electron microscopy)

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ABSTRACT Previous physiological evidence has shown that the receptor neuron of the slowly adapting stretch receptor organ of crayfish receives synapses from three inhibitory axons, while the receptor muscle is innervated by both excitatory and inhibitory axons. Fine structural studies have indicated that after certain preparative procedures synaptic vesicles in the inhibitory terminals on dendrites of the receptor neuron appear small and elongate, while those in the excitatory terminals on the receptor muscle are round and larger. This study describes a new synapse between two inhibitory nerve endings on the receptor neuron. One axon, containing small elongate vesicles, forms a presynaptic chemical contact with another morphologically similar axon that, itself, presumably releases inhibitory transmitter onto the receptor neuron. A second type of presynaptic axo-axonic synapse, analogous to one previously described in another crustacean muscle, was also found between presumed inhibitory and excitatory nerve terminals on the receptor muscle. Thus, the stretch receptor has a relatively complex organization with a morphological basis for two types of presynaptic inhibition: one on excitatory terminals and the other on inhibitory terminals.

The simplest synaptic inhibitory mechanism in the nervous system is secretion by a presynaptic nerve terminal of a chemical that reduces the ability of the postsynaptic cell to respond to excitation by another nerve. There exists, in addition, physiological evidence for an inhibition in which one nerve terminal prevents release of an excitatory transmitter from another nerve terminal onto a postsynaptic cell. This type of interaction, called presynaptic inhibition, has been described in crustacean neuromuscular junction (1) and in vertebrate central nervous system (2, 3).

In their electron microscopical study of the opener muscle of crayfish claws, Atwood and Morin (4) found in the vicinity of neuromuscular junctions the apparent morphological basis for presynaptic inhibition by describing a typical chemical synapse between the inhibitory and motor nerve terminals. In this preliminary report, we will describe two types of axo-axonic synapses in the stretch receptor organ of crayfish. One occurs on the motor nerve terminal supplying the receptor muscle; it is similar to that reported by Atwood and Morin (4) and corresponds to their presynaptic inhibitory synapse. The other is found between two morphologically similar terminals on the stretch receptor neuron. It is of particular interest since it suggests the existence of presynaptic inhibition at an inhibitory synapse, which has not previously been described in any nervous system.

METHODS

The abdominal stretch receptor organs of crayfish (Procambarus) were exposed in van Harreveld’s physiological solution (5), fixed with phosphate-buffered 1.6% glutaraldehyde (pH 7.4), washed with phosphate buffer, and postfixed with phosphate buffered 1% osmium tetroxide. In most specimens all these solutions were isosmotic with the physiological solution (440 milliosmols). In addition, some specimens were fixed and/or washed with solutions that were made either hypertonic (880 and 1320 milliosmols) or hypotonic (145 milliosmols) to the physiological solution by alteration of the buffer concentration. After dehydration, specimens were gradually infiltrated with epon. During this stage, the slowly adapting receptor organ, easily distinguishable from the fast one by its location and structural characteristics, was separated under a dissecting microscope and embedded. The specimens were then cut with a Porter-Blum MT2-B or an LKB ultramicrotome, and examined with a Philips 300 or an AE12B electron microscope.

RESULTS AND DISCUSSION

The anatomy of crustacean stretch receptor organ and its innervation have been described by Alexandrowicz (6) and Florey and Florey (7) and is shown in the diagram (Fig. 1A). The slowly adapting stretch receptor of crayfish consists of a narrow muscle extending between segments of the exoskeleton in the dorsal region of the abdominal and posterior thoracic segments. In the receptor muscle are embedded the dendrites of a receptor neuron whose axon extends to the central nervous system. The receptor neuron dendrites are innervated by three efferent fibers of different diameters (I₁, I₂, and I₃ in Fig. 1A or, in the terminology of Alexandrowicz, thick and thin accessory fibers and fiber 2). One or possibly two of these (I₁ and I₂) give off branches that innervate the receptor muscle which also receives separate motor fibers.

There is now good physiological evidence (8–10) that all efferent innervation of the receptor neuron is inhibitory with the different inhibitory fibers having different degrees of effectiveness. In addition, all synapses on dendrites of the

Abbreviations: SVT, small-vesicle terminal; LVT, large-vesicle terminal.

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receptor neuron contain small elongate vesicles (11). On this basis, the presence of small elongate vesicles in nerve terminals has been used as one criterion for distinguishing inhibitory from excitatory synapses in crustacean muscle.

**Axo-dendritic and neuromuscular synapses**

In agreement with the studies of Uchizono (11), Atwood and Morin (4), and Nadol and de Lorenzo (12) we have found that the synaptic terminals on the slowly adapting stretch receptor neuron, when fixed and washed in isotonic solutions, contain elongate synaptic vesicles about 330–370 Å in diameter (Figs. 2 and 4). (Diameter is defined here as the geometric mean of the maximum and minimum diameters of each vesicle.) We will hereafter refer to this type of terminal, which probably mediates inhibition of the receptor neuron, as SVT (small-vesicle terminal). These are distinguishable from a second type of terminal that was seen on the stretch receptor muscle only. When prepared under isotonic conditions, it contains round vesicles about 410–450 Å in diameter. The second type of terminal probably forms an excitatory neuromuscular junction (13, 14) and we will call it LVT (large-vesicle terminal). SVTs were also found in synaptic contact with the receptor muscle, and these probably correspond to inhibitory synapses on the receptor muscle (14). Evidence that SVTs and LVTs correspond to inhibitory and excitatory synapses on another crustacean muscle is provided by recent experiments of Atwood et al. (15) in which they selectively depleted SVTs of vesicles by stimulation of the inhibitory axon and depleted LVTs of vesicles by stimulation of the excitatory axon in claw-open muscle.

**Axo-axonic synapses**

Atwood and Morin (4) described axo-axonic synapses in the crayfish claw opener in which SVTs were presynaptic to LVTs, which in turn made a chemical synapse with the muscle. They considered this to be the morphological correlate of presynaptic inhibition of the excitatory innervation of claw-opener muscle. We have observed an analogous relationship in which SVTs are presynaptic to LVTs making a synapse with the receptor muscle (Fig. 4). The diagram in Fig. 1C represents this contact.

In addition to this type of axo-axonic synapse, we have observed a second kind which has not hitherto been seen. In this contact one SVT is presynaptic to another SVT, which in turn is presynaptic to dendrites of the sensory receptor neuron, as shown in Figs. 2 and 3. These contacts were found only in the dendritic regions of the receptor neuron and are characterized as chemical synapses by aggregates of small elongate synaptic vesicles and dense material next to the membrane of the presynaptic axon (white arrows in Figs. 2 and 3) and an accumulation of dense material just inside the membrane of the adjacent postsynaptic axon. The postsynaptic element of this pair, in turn, contacts another nerve process (D in Fig. 2) by another typical SVT synapse (black arrows in Figs. 2 and 3). This third element does not contain synaptic vesicles but usually contains neurotubules, glycogen granules, and mitochondria and is often clearly continuous with a large dendritic process of the receptor neuron. The diagram in Fig. 1B represents this type of axo-axonic contact that was observed several times in each of several animals.

Not only did the pre- and postsynaptic axons at the SVT–SVT synapse resemble each other in the type of vesicles they contained, but, in addition, both were found in two cases to be presynaptic to a single dendrite of a receptor neuron in the same thin section. Since inhibitory responses have been positively identified in the receptor neuron, while excitatory synaptic effects have not been recorded, one can be confident that both elements of the SVT–SVT synapse are inhibitory to the

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*Fig. 1. Schematic drawings of the slowly adapting stretch receptor. (A) Diagram showing the innervation of the slowly adapting stretch receptor. The dendrites of the receptor neuron receive three inhibitory axons of different diamters. In the nomenclature of Alexandrowicz (6), the thick (I₁) and intermediate-size (I₂) axons are called the thick and thin accessory fibers, respectively. The thinnest inhibitory axon (I₃) is named fiber x. The receptor muscle receives one or two of these inhibitory axons (the thick accessory fiber and probably fiber x) in addition to other motor axons. (B) Schematic drawing of an axo-axonic synapse found in the dendritic region of the receptor neuron (area B in part A). This type of axo-axonic synapse is formed by two small-vesicle terminals. The postsynaptic terminal of this synapse makes synaptic contact with the dendrite of the receptor neuron. (C) Schematic drawing of an axo-axonic synapse found on the receptor muscle (area C in part A). This type of axo-axonic synapse is formed by a small-vesicle terminal containing small elongate vesicles and a large-vesicle terminal containing large round vesicles. The large-vesicle terminal, the postsynaptic component of this synapse, makes synaptic contact with the receptor muscle.*
Fig. 2. Electron micrograph showing an axo-axonic synapse (white arrow) formed between two small-vesicle terminals, which are considered to be inhibitory. Procambarus fixed and washed with solutions isosmotic with the physiological solution. The postsynaptic terminal of this axo-axonic synapse in turn makes synaptic contact (black arrows) with a dendrite (D) of the receptor neuron. Both small-vesicle terminals contain small elongate synaptic vesicles (sv). nt, neurotubules; ce, cored vesicles; db, dense bodies. ×34,600.
receptor neuron. A further indication of the similarity of function between the two synaptic components was obtained by varying the conditions of fixation. It has been shown repeatedly (16–18) that the size and shape of synaptic vesicles can be changed by alteration of fixative components and osmolarity, and that controlled use of these changes might be helpful for distinguishing different types of synapses. We found in our preparation that when specimens were washed with a hypertonic solution (145 milliosmols) after fixation, vesicles in SVTs and LVTs both appeared round, although those in the SVTs were smaller. On the other hand, when the specimens were washed in a hypertonic solution (1320 milliosmols) after fixation the vesicles in both types of terminals appeared elongate, but still of different sizes. In all cases of the SVT-SVT synapses, the size and shape of the synaptic vesicles were affected similarly by the toxicity of the wash used. Thus, both components of the SVT-SVT synapse share anatomical and certain physiological similarities.

Our structural evidence suggests that synapses occur between inhibitory terminals on the receptor neuron. Physiological studies are required to clarify the interaction among inhibitory axons that are efferent to the receptor neuron, particularly since no physiological examples are known in which the release of inhibitory transmitter from a terminal is influenced by another axon.

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