THE FINE STRUCTURE OF A RECTIFYING ELECTROTONIC SYNAPSE

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ABSTRACT

The synapses between the lateral giant axon and the giant motor axon found in the abdominal ganglia of the ventral nerve cord of the crayfish Procambarus clarkii are electronic. The junctional membrane rectifies, favoring impulse transmission from lateral giant fiber to giant motor fiber. This rectifying electronic junction consists of closely apposed membranes indistinguishable from ordinary arthropod gap junctions. The apposed membranes contain intramembrane particles that are ~ 12.5 nm in width. These particles have a central depression and are arranged in a loosely ordered array with a center-to-center spacing of about 20 nm.

The only obvious morphological evidence of asymmetry is the presence of vesicles (about 80 nm in diameter) in the cytoplasm adjacent to the junctional region of the presynaptic lateral giant fiber. Vesicles are not present in the adjacent cytoplasm of the postsynaptic giant motor fiber; however, mitochondria and smooth tubular endoplasmic reticulum are more frequent in the cytoplasm of the giant motor fiber.

KEY WORDS cell-to-cell communication · rectifying electrotonic junction · gap junction · freeze-fracture · electron microscopy

Although most electrotonic junctions behave as fixed resistances connecting coupled cells, a few instances are known in which the junctions rectify. Furshpan and Potter (9) established that transmission at the giant motor synapse of the crayfish is electrical with rectification. Conduction is essentially a one-way transmission from lateral giant fiber to giant motor fiber. When the potential in the lateral giant axon (presynaptic) is positive relative to the potential in the giant motor fiber (postsynaptic), junctional resistance is low. At rest, and when the motor fiber is more positive, junctional resistance is high. Thus orthodromic transmission is favored over antidromic.

Robertson (28) and Stirling (30) have suggested that the junctional membranes in this synapse form intimate contact. Our preliminary findings (12, 15) indicate that these rectifying junctions may be similar morphologically to the more common linear electrotonic synapses. To positively identify topologically the location of these junctions, nerve cords were initially prepared for scanning electron microscopy. Further studies of this synapse on both sectioned and freeze-fracture preparations were carried out. We were unable to distinguish any structural differences between the

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membranes of the rectifying junctions and those of the linear electronic synapses in the septa between segments of the lateral giant axon in the crayfish. Clusters of large (~80 nm) vesicles were found in the cytoplasm next to the gap junction only in the lateral giant fiber side (presynaptic).

**MATERIALS AND METHODS**

**Sectioned Material**

The ventral nerve cord of the crayfish, Procambarus clarkii, was fixed in situ by immersion in glutaraldehyde-H$_2$O$_2$ overnight (25). The nerve cord was then dissected out and either postfixed in 1% O$_2$O$_2$ or prepared for freeze-fracture. Tissue was dehydrated in an ascending series of ethanol and embedded in Epon 812. Thick sections (~2 μm) were cut and stained with toluidine blue and examined in the light microscope. When the appropriate junctions were identified, thin sections were cut on an ultramicrotome and stained with uranyl acetate and lead citrate. Some nerve cords were presoaked for 1 h in 4% ionic lanthanum (pH 7.2) before fixation.

**Freeze Fracture**

Small pieces containing the third root of the abdominal ganglia were dissected from glutaraldehyde-H$_2$O$_2$-fixed ventral nerve cord and soaked in 30% glycerol-Ringer solution for not more than 30 min. The specimens were then frozen in liquid freon 22 and fractured in a Balzers 301 apparatus (Balzers High Vacuum Corp., Santa Ana, Calif.). The replicas were prepared by the evaporation of 1.5 nm of platinum and 20 nm of carbon. The tissue was digested in Clorox (Clorox Co., Oakland, Calif.), and the replicas were picked up on formvar-coated single-slotted grids. The revised nomenclature of Branton et al. (4) was used for identifying membrane fracture faces.

**Scanning Electron Microscopy**

The connective tissue sheaths on the dorsal surface of the nerve cord were carefully dissected so that the contours of the giant motor fiber on the surface could be seen with the high power of the dissecting microscope. Segments of nerve cord were fixed in the usual manner, dehydrated, and critical-point dried with liquid CO$_2$ as the intermediate fluid. The tissue was oriented onto the specimen platform so that the crossing of the giant motor fiber in the dorsal surface of the cord could be visualized with the scanning electron microscope. The preparation was sputter-coated with gold-palladium.

**RESULTS**

The giant-motor-fiber forms electrotonic synapses which rectify with both the lateral giant axon and the medial giant axon (9). The giant motor fiber can be seen on the surface of the cord (Fig. 1), juxtaposed above the medial giant fiber. These junctions are located just anterior to the exit of the third root of the ganglia from the ventral nerve cord (Fig. 1) (9, 16). As the giant motor fiber passes between the lateral giant and the medial giant axon, small finger-like processes 2.5–3 μm thick extend from a branch of the giant motor fiber to the lateral giant axon to form the synaptic contacts (Fig. 2).

Fig. 3 also shows a profile of a single finger-like extension of the giant motor fiber making synaptic contact with the lateral giant fiber, as seen in the electron microscope. The region of synaptic contact is characterized by closely apposed membranes, the presence of vesicles in the lateral giant fiber, and the presence of mitochondria in the giant motor fiber. The closely apposed membranes are separated by a gap of about 4 nm (Fig. 4), which is rather large when compared with that of other electrotonic junctions (10, 11, 17, 21). When the rectifying junction is stained with lanthanum and viewed en face, facets with a periodicity of about 20 nm are observed (Fig. 5). These clear areas, which exclude the extracellularly applied lanthanum, are a negative image of intercellular particles visualized in freeze-fracture preparations. (cf. Figs. 6–10).

Vesicles, approximately 80 nm in diameter, are present in the cytoplasm clustered close to the junctional (presynaptic) plasma membrane of the lateral giant fiber (Fig. 3). They are never found in the cytoplasm of the junctional region of the giant motor axon (the postsynaptic fiber); however, mitochondria and elements of smooth tubular endoplasmic reticulum are more frequent.

The lateral giant fiber makes other synaptic contacts in addition to those on the giant motor fiber. However, these additional synapses are found within the neuropil of the abdominal ganglia (16, 29, 32, 33) and not at the site identified in Fig. 1.

The location of the rectifying synapse, at the exit of the third root (Fig. 1), was isolated quite easily by dissection. In this way we eliminated any possible confusion with the other synaptic contacts that are present in the neuropil of the ganglion. In the isolated region of the third root, the only synaptic contacts on the lateral giant fiber that were observed by both light microscopy and electron microscopy were made by the giant motor fiber (Figs. 2, 3). We did not observe any junctional contacts between passing axons and the lateral giant fiber at these sites.
FIGURE 1 Scanning electron micrograph of a segment of the ventral nerve cord of a crayfish showing the isolated region of the third root after being dissected from the abdominal ganglion. The lateral giant fiber (LGF)-giant motor fiber synapse is located just anterior to the exit of the third root (in the area of the arrow). MGF = medial giant fiber. × 40. The inset shows the intact nerve cord before the isolation at the LGF-GMF synapse (arrow). × 10.

FIGURE 2 Light micrograph of a section in the region of synaptic contact in the third root of the crayfish abdominal nerve cord. Finger-like processes (arrow heads) extend from the giant motor fiber (GMF) to the lateral giant axon (LGF). Section 2 μm thick, stained with toluidine blue. × 1,500.
Observation of freeze-fracture replicas at low magnification permits relatively easy identification of the lateral giant fibers and the medial giant fibers because of their position and large size when compared with the other axons. The membrane and junctional features of the plasmalemma of the lateral giant fiber in the replicas were correlated with the morphological characteristics observed in thin sections, and in the dissected region no other gap junctions are formed with the lateral giant fiber. The careful application of the above-stated criteria thus permits positive identification of the lateral giant fiber-giant motor fiber synapse in the freeze-fracture replicas, as seen in Fig. 6. This micrograph shows an obliquely fractured junctional region, and thus demonstrates the asymmetrical distribution of vesicles. The vesicles are clustered close to the junctional membrane in the presynaptic fiber while mitochondria are seen in the cytoplasm of the postsynaptic fiber. Fig. 6 shows the P face of the lateral giant fiber (presynaptic) and the E face of the giant motor fiber (postsynaptic) while subsequent replicas are from the E face of the lateral giant fiber and from the P face of the giant motor fiber.

In Fig. 7, a single process about 3 μm wide is seen making synaptic contact with the lateral giant fiber (LGF). The characteristic fingerlike processes of the giant motor fiber that make synaptic contact with the lateral giant fiber measure about 2.5-3 μm in width (Fig. 2). Not only in terms of size but also by location, this junctional complex is identified as the lateral giant-to-giant motor synapse. Clusters of vesicles (arrows) are seen in the axoplasm of the lateral giant fiber. Mitochondria (m) and smooth tubular endoplasmic reticulum characteristically are found in the axoplasm of the postsynaptic giant motor fiber. In Fig. 7, if a thin section were to be made along the dotted line a-b, its profile would be very similar in appearance to Fig. 3.

The intramembrane particles which characterize the electronic junction adhere almost exclusively to the E face of the junctional membranes, with the complementary pits being observed on the P face (Fig. 7, inset). The intramembrane particles of the rectifying junction measure ~12.5 nm in diameter (Figs. 7-9). These particles can often be observed to have a central depression or dimple when viewed at high magnifications (Fig. 8, inset).

The particles are arranged in a loosely ordered array with a center-to-center spacing of about 20 nm (Figs. 7, 8). The degree of order within this loose array becomes readily apparent if Fig. 8 is tilted almost horizontally and viewed in the direction of the large arrows.

In thin sections, one may observe “connections” between the vesicles and the membranes of the rectifying junction (Fig. 4 at arrows). Fig. 9 (inset) shows an apparent continuity (at arrowhead) of a gap junction and a vesicle in a freeze-fracture replica.

Because the fracture plane follows a very irregular path, it is usually not possible to measure the height of the intramembrane particles. However, a direct measurement of particle height is possible in Fig. 10. In this instance, a fortuitous tear in the replica occurred through the junctional region, and the edges have curled away from the electron beam, thus providing a side view of the particles from within the plane of the replica. The particles are approximately 10 nm in height as measured from the surface of the E face. The particles are calculated to protrude into the cytoplasm for about 5 nm after one subtracts 5 nm for collapsed lipid and the protoplasmic half of the membrane (5).

DISCUSSION

It has been suggested that the intramembrane particles observed in freeze-fracture replicas of gap junctions provide the pathway by which intercellular communication is accomplished (1, 18, 21, 22). We had hypothesized that the rectification or regulation of ionic flow might be mediated by the gap junctional channels and that this mediation might be somehow reflected in a morphological dissimilarity in the intramembrane particles of the rectifying junction. This is not the case, however, as no apparent morphological differences were found between the particles or their arrangement on either side of this synapse when compared with the nonrectifying gap junctions (23). Furthermore, our studies show that the junctional membranes are separated by ~4 nm, similar to that demonstrated at gap junctions between segments of the lateral giant axons (19). This does not rule out the possibility that the particles do in fact regulate the ionic flow and that dissimilarities are not detectable with the present techniques.

In earlier permeability studies, we reported that intracellularly injected fluorescein crosses the septal gap junctions of the crayfish, as in a number of other nonrectifying gap junctions (2, 19, 20). In contrast, our recent, still preliminary, studies have
failed to show fluorescein crossing the lateral-to-giant fiber junction in either direction, although electrical transmission was normal (14). However, as the junctional membrane is of high resistance at rest, the permeability should be studied during stimulation that lowers the junctional resistance.

The only obvious morphological asymmetry at the rectifying junction is the distribution of the vesicles found only in the presynaptic lateral giant fiber. Frequently, clusters of mitochondria are present in the postsynaptic giant motor fiber. Vesicles are often found at electrotonic synapses when one or both junctional processes are axons. In the septal axo-axonic electrotonic synapses of the lateral giant fibers in the ventral nerve cord of the crayfish, a symmetrical distribution of vesicles is present next to the gap junction (19, 20).

It is known that vesicles at chemically transmitting synapses can bind calcium ions (13, 27). Peracchia and Dulhunty (24) have suggested that the large vesicles present near electrotonic junctions may bind the calcium ions which enter the junctional region during an action potential. Conversely, Peracchia and Dulhunty further suggested that in the postsynaptic giant motor fiber, where there are no vesicles, the increase in calcium ion concentration may block electronic transmission (24). However, it is difficult to explain how such a block would be unidirectional.

The presence of clusters of vesicles next to the presynaptic side of the rectifying junction is not analogous to what has been described as morphologically mixed synapses in the vertebrate central nervous system (3, 26). As can be readily seen in Figs. 3, 4, 7, and 9, the vesicle clusters are found in the cytoplasm focally at the site of junctional specialization, while at the vertebrate mixed synapses vesicle clusters usually face the presynaptic cytoplasmic dense material of the chemical synapse and not the area of gap junctional specialization.

Understanding the function of the vesicles is made even more complex by the apparent physical continuity between the vesicles and the intramembrane particles (Figs. 4, 9). Peracchia (22, 23) first observed the continuity between the vesicles and the intramembrane particles in thin sections of the axo-axonic synapses in the septa of the lateral giant fibers. He postulated that it would therefore be possible to have an intercellular exchange of molecules by membrane-bounded compartments. The asymmetrical distribution of the vesicles at the rectifying synapse leads obviously to the speculation that the vesicles are somehow responsible for the rectification; however, it is very difficult to envision a mechanism by which the presence of a vesicle and (or) its continuity with the junctional particles is necessary for the flow of ions through the electrotonic junctions. Thus, at the present time the function of the vesicles at electrotonic synapses remains unclear.

It is interesting to note that the particles observed in freeze-fracture preparations at both the gap junction in the septa of the lateral giant axons and the lateral giant fiber to giant motor fiber...
FIGURE 7 Electron micrograph at low magnification of a portion of freeze-fracture replica showing a single finger-like extension of the giant motor fiber (GMF) making synaptic contact with the lateral giant fiber (LGF). At arrowheads, three clusters of vesicles in the cytoplasm of the lateral giant fiber characterize the presynaptic side of the junctional complex. A hypothetical thin section along dotted line \( a-b \) would be very similar in appearance to the section shown in Fig. 3. \( \times 18,000 \). Inset shows a higher magnification of the area outlined. The particles adhere to the \( E \) face (Ef) of the synaptic membrane while the complementary pits are present on the \( P \) face (Pf) of the membrane complex. Note the orderly arrangement of the pits. \( \times 88,000 \).
adhere to the $E$ face rather than the $P$ face as in most other gap junctions. It has been noted that this is a characteristic feature of gap junctions in arthropods (8, 10, 23). Recently, Wood (31) has found that the particles at gap junctions in Hydra also adhere to the $E$ face. He has suggested that this cleaving property of the gap junction particles, in Hydra at least, may indicate strong intercellular
adhesive forces, since fragments of cytoplasm were found to cover the junction. Furthermore, in the majority of cases the fracture plane at the gap junction passes through the presynaptic lateral giant fiber plasma membrane and not through the postsynaptic giant motor fiber membrane. However, this observation may be the result of our sampling since the presence of vesicles in the axoplasm makes those junctions easier to locate. The observation that the intramembrane parti-
cles may actually protrude into the cytoplasm for about 5 nm (Fig. 10) is consistent with the concept of a hydrophilic channel linking the cytoplasm of the two electrically coupled cells. Utilizing thin sections, Peracchia (22) has observed globules, which presumably are the intramembrane particles, in the nonrectifying electrotonic junction which also protrude into the cytoplasm 2-3 nm. Thus, the possibility that gross plastic deformation of the particles may have occurred during the fracturing process would seem to be very remote. The junctional particles protrude into the cytoplasm from the E face and, therefore, the central depression seen in the junction particles (Fig. 8) may represent the cytoplasmic "opening" of the hydrophilic channel (23).

There is a high degree of long-range order within the junction which can be observed if Fig. 8 is tilted almost horizontally and viewed along major axes (large arrows). This long-range order is at first not apparent because of the short-range disorder among the particles (6). The short-range disorder among the particles may be the result of plastic deformation of the particles. The complementary pits seem to exhibit a higher degree of order than the particles perhaps because they are not as susceptible to deformation (7) (see Fig. 7 inset). The junctional particles of this synapse may be in a highly ordered hexagonal array in the intact membranes.

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