SIMILARITY OF JUNCTIONS BETWEEN PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM IN MUSCLE AND NEURONS

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ABSTRACT

The structure of membranes at junctions between the plasma membrane and underlying cisterns of endoplasmic reticulum in amphioxus muscle and mouse cerebellar neurons was studied using the freeze-fracture technique. In amphioxus muscle, subsurface cisterns of sarcoplasmic reticulum form junctions with the surface membrane at the level of the sarcomere I bands. On the protoplasmic leaflet of the sarcolemma overlying these junctions were aggregates of large particles. On the protoplasmic leaflet of the membranes of cerebellar basket, stellate, and Purkinje cells there were similar aggregates of large particles. In both tissues, the corresponding external membrane halves had arrays of pits apparently complementary to the aggregates of large particles. Cross fractures through junctions showed that the particle aggregates in neuronal and muscle membranes were consistently located over intracellular cisterns closely applied to the plasma membrane. Thus, a similar plasma membrane specialization is found at subsurface cisterns in mammalian neurons and amphioxus muscle. This similarity supports the hypothesis that subsurface cisterns in neurons, like those in muscle, couple some intracellular activity to the electrical activity of the plasma membrane.

Junctions between the surface or transverse tubule membrane and the endoplasmic, or sarcoplasmic, reticulum of muscle are thought to mediate excitation contraction coupling (12, 34, 39). Junctions between the surface membrane and regions of endoplasmic reticulum (ER), known as subsurface cisterns, are also present in neurons (38). Their similarity to the junctions in muscle has been noted (27, 38), but the functions of subsurface cisterns in neurons are unknown. We have used the freeze-fracture technique to compare the structure of the membranes participating in these junctions in striated muscle from the chordate, amphioxus, to their structure in neurons of the mammalian cerebellar cortex. The morphologic similarities apparent in thin sections are paralleled by similarities in the arrays of membrane particles revealed by freeze-fracturing the membranes at these junctions. These further similarities lend support to the suggestion (19, 20, 38) that subsurface cistern junctions may function in neurons, as in muscle, to couple excitatory events at the surface membrane with intracellular activities. A preliminary report of our results has been presented (25).

MATERIALS AND METHODS

Amphioxus Branchiostoma californiensis were obtained from Pacific Bio-marine Supply Co., Venice, Calif., and maintained in cold natural seawater for short periods

before use. Animals were decapitated, and the remaining body was skinned and cut into segments which were immersed in fixative containing 2.5% glutaraldehyde, 0.1 M cacodylate buffer, pH 7.6, and 0.8 M sucrose. After fixation for 1 h, the tissue was rinsed in buffer containing sucrose, cut into small cubes suitable for freeze-fracturing, and soaked in 20% glycerol in 0.05 M cacodylate buffer in preparation for freezing. Adult C57BL/6J mice were anesthetized with intraperitoneal chloral hydrate and perfused through the heart with fixative containing 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate or 0.08 M cacodylate buffer at pH 7.2–7.3 and 37°C for 5–20 min. The cerebellum was rinsed in buffer, chopped into slabs of uniform thickness with a Smith-Farquhar tissue chopper, and then equilibrated with 20% glycerol in 0.05 M buffer.

All tissues were frozen on gold disks in Freon 22, and freeze-fractured in a Balzers 360 M apparatus (Balzers AG, Balzers, Lichtenstein) equipped with an electron beam gun. Photographic prints of replicas are positives so that regions of platinum deposition appear dark. Pieces of tissue for thin sectioning reserved from most experiments were postfixed in osmium tetroxide and stained with 1% uranyl acetate in acetate buffer. Examinations of junctions in thin sections was facilitated by using AEI-802 or JEM 100B electron microscopes equipped with tilt stages.

RESULTS

Amphioxus Muscle

The sarcoplasmic reticulum of amphioxus muscle forms areas of association with the surface membrane, which resemble the junctions between the sarcoplasmic reticulum and transverse tubule or surface membranes in other muscle. In amphioxus, cisterns of sarcoplasmic reticulum form junctions with the surface membrane in the region of the I bands, and these junctions are characterized by a paralleling of the surface and sarcoplasmic reticulum membranes over the length of the cistern. In the gap (roughly 20 nm wide) between the apposed membranes is an amorphous material sometimes appearing as periodic densities. In material prepared as described above, the sarcoplasmic cisterns protrude above the level of the contractile material, causing the surface membrane to bulge at the junctional areas (reference 17; see also Fig. 1).

The sarcomeres and, therefore, the sarcoplasmic reticulum cisterns of adjacent muscle lamellae also tend to be in register, and these features make identification of the junctional areas in freeze-fracture images unmistakable (Figs. 2–4). Clusters of intramembrane particles averaging 90

Å in diam fracture with the protoplasmic leaflet of the surface membrane overlying the junctions with the sarcoplasmic reticulum cisterns. These particles are distinguished by their uniform diameter and large size (Figs. 2–3). Away from the junctions the protoplasmic leaflet of the surface membrane has scattered particles in at least two distinctive size ranges: a few particles comparable in size to those in clusters at the junctions, and numerous smaller particles (Figs. 2 and 3). In the external leaflet of the surface membrane overlying junctions are clusters of pits probably corresponding to the particles which cleave with the protoplasmic leaflet (Fig. 3). Fractures passing through the sarcoplasmic reticulum membrane at its apposition to the surface were rare, but on the nearby sarcoplasmic reticulum membrane are scattered large particles that fracture with the protoplasmic leaflet of the membrane while its luminal leaflet has pits which are, presumably, imprints of these particles (Figs. 2–4).

Mouse Cerebellum

Subsurface cisterns in cerebellar Purkinje cells are formed by flattened sacs of ER closely applied to the neuronal plasmalemma (41, 42), forming junctions which are very similar to the sarcoplasmic junction described above (Fig. 5). These junctions in the cerebellum are characterized by a densely packed fuzz lying in the roughly 20-nm-wide gap between the outer cisternal membrane and the plasma membrane. Careful tilting of sections through the junction showed that the fuzzy material is distributed uniformly along the cleft, punctuated by a faint periodicity. The inner and outer cisternal membranes are closely applied to each other over the region coextensive with the junction, and in this area their luminal membrane leaflets are separated by only 2–5 nm. The lumen of the annular region of the cistern which surrounds the junction is much larger. Ribosomes are occasionally associated with the cisternal membranes, and mitochondria are often applied to the innermost aspects of the cisterns (Fig. 5).

The freeze-fractured plasma membranes of cell bodies and proximal dendrites of basket, stellate, and Purkinje cells are characterized by circular aggregates of particles on their protoplasmic leaflets (Fig. 6). Particles in such aggregates are uniformly larger than those scattered over the rest of the plasma membrane (Figs. 6 and 7). These aggregates average 0.5 μm in diam. and have particles clustered in them at a concentration of ap-
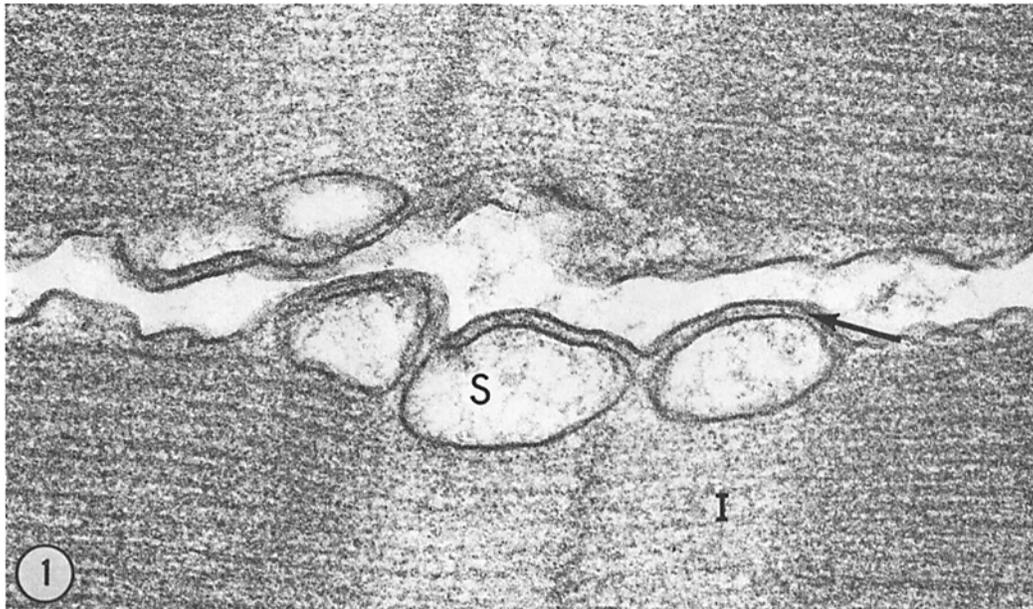
FIGURE 1 Longitudinal section of amphioxus skeletal muscle. Cisternae of sarcoplasmic reticulum (*S*) abut the plasmalemma, producing hemispherical bulges on the muscle surface which are aligned with the I bands (*I*). At these bulges, the cisternal membrane is parallel with the muscle plasmalemma and a fuzzy material (arrow) lies in the gap between the two membranes. × 90,000.

prox. 2,000 per μm^2 (Fig. 7). Neither particle size nor distribution is precisely uniform across the aggregate, but no consistent pattern of particle distribution was evident. Rough estimates of the area occupied by this type of aggregate indicate that they cover approx. 2% of the surface of cell bodies. Fractures exposing the external leaflet of the plasmalemma had areas pocked by minute pits which, because they are similar in shape and distribution to the particle aggregates, were taken to be imprints of them (Fig. 8).

Although the size, shape, and distributions of subsurface cistern junctions in thin sections corresponded well to the distribution of the particle aggregates described above, further evidence was required to prove that these particle aggregates corresponded to subsurface cistern junctions. Occasionally, cross fractures in the plasma membrane at a junction permitted the underlying membranes to be seen. In every instance, flattened sacs of membrane, or cisterns, lay under these aggregates (Figs. 9–10).

Fractures through cistern membranes, though rare, were sufficient to show certain specializations. In some instances, pits in the luminal leaflet of the cisternal membrane were confined to the junctions, indicating that the frequency of large

particles must be greater in this region (Fig. 10). A few direct looks at small patches of the protoplasmic leaflet of this membrane indeed showed many large particles (Fig. 10), but this information does not indicate whether their distribution in any way corresponds to the distribution of the large particles in the overlying plasmalemma. The protoplasmic leaflet of the rest of the cisternal membrane was also characterized by numerous particles, though these were not so large as those in the protoplasmic leaflet of the plasmalemma over the cisternal junction (Fig. 9).

Astrocyte processes usually are opposite sites of subsurface cisterns in neuronal perikarya or proximal dendrites (Figs. 5 and 10). The membrane of these astrocyte processes contains orthogonal particle aggregates, termed assemblies (26), but there is no particular specialization of the astrocyte membrane in register with the neuronal subsurface cistern (Fig. 10). Thus, the aggregates of large particles in the surface membrane overlying subsurface cisterns do not correspond to a specialized junction with the adjacent cell. Subsurface cisterns in Purkinje cells also occasionally lie under the margin of a basket cell axon bouton, but never appear to occupy the entire postsynaptic area.
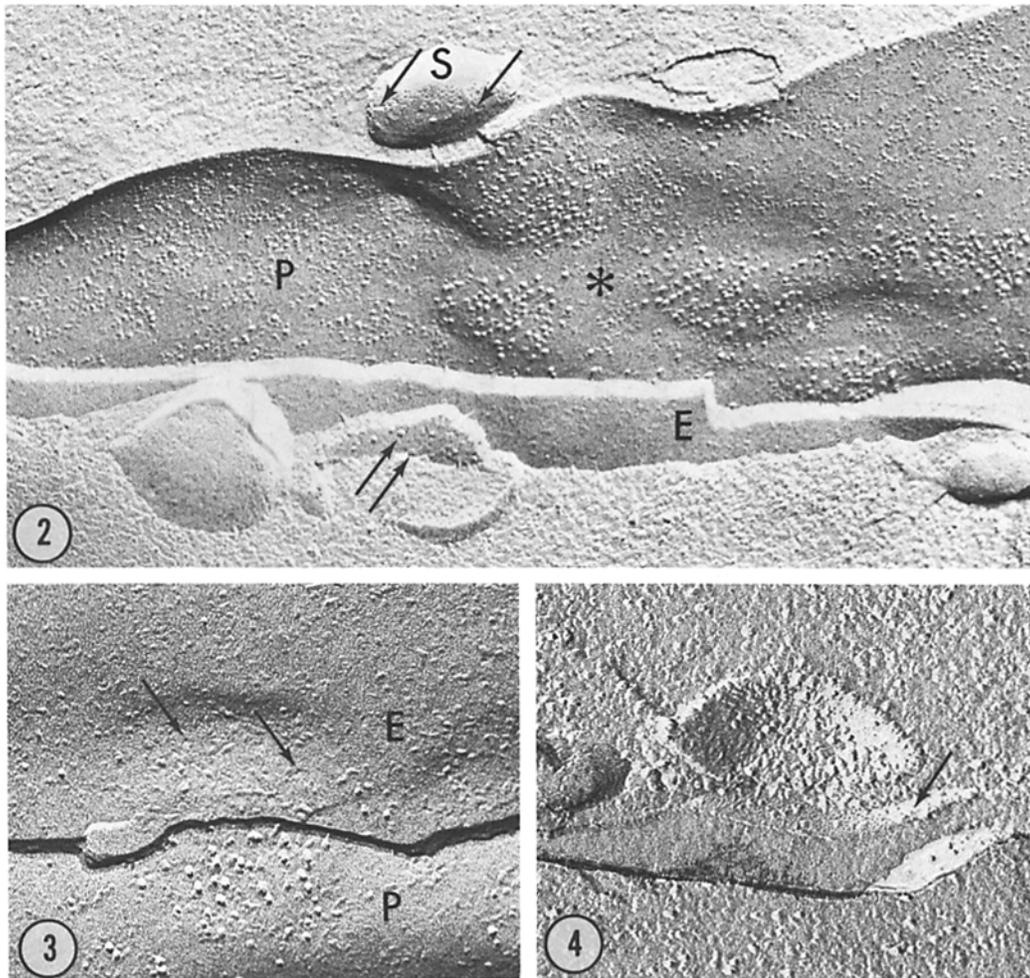
FIGURE 2   Freeze-fractured plasmalemmas of two adjacent striated muscle lamellae of amphioxus. The protoplasmic leaflet (P) of the plasmalemma of one muscle cell and the external membrane leaflet (E) of the adjacent one are exposed by this plane of fracture. Bulges on the protoplasmic leaflet of the plasmalemma (near asterisk), where it lies adjacent to cisterns of sarcoplasmic reticulum (S), are marked by aggregates of particles uniformly larger than most particles on the adjacent membrane. The cisternal membrane is also marked by large particles on its protoplasmic leaflet where it faces the plasmalemma (lower arrows) and by pits in its luminal leaflet (upper arrows) which are presumably complementary to the particles. × 75,000.

FIGURE 3   Freeze-fractured plasmalemmas of two adjacent muscle lamellae from amphioxus in the region of junction between the sarcoplasmic reticulum and the plasmalemma. Pits (arrows) are present over the bulged region of the external leaflet (E) of the plasma membrane. These are apparently complementary to particle aggregates found on the protoplasmic membrane leaflet (P). × 100,000.

FIGURE 4   Particles on the protoplasmic leaflet of a cistern of sarcoplasmic reticulum in amphioxus muscle. The plasma membrane is below (arrow) so that these particles lie in a region of the cistern outside its junction with the plasma membrane of the muscle fiber. Platinum arrived from right. × 100,000.

DISCUSSION

Junctions between the sarcoplasmic reticulum and transverse tubule membranes are visible in thin sections of muscles from many different animals. Although there are minor differences such as width of the gap between the two membranes or the extent of the fuzz in it (e.g., 13, 14, 17, 23),
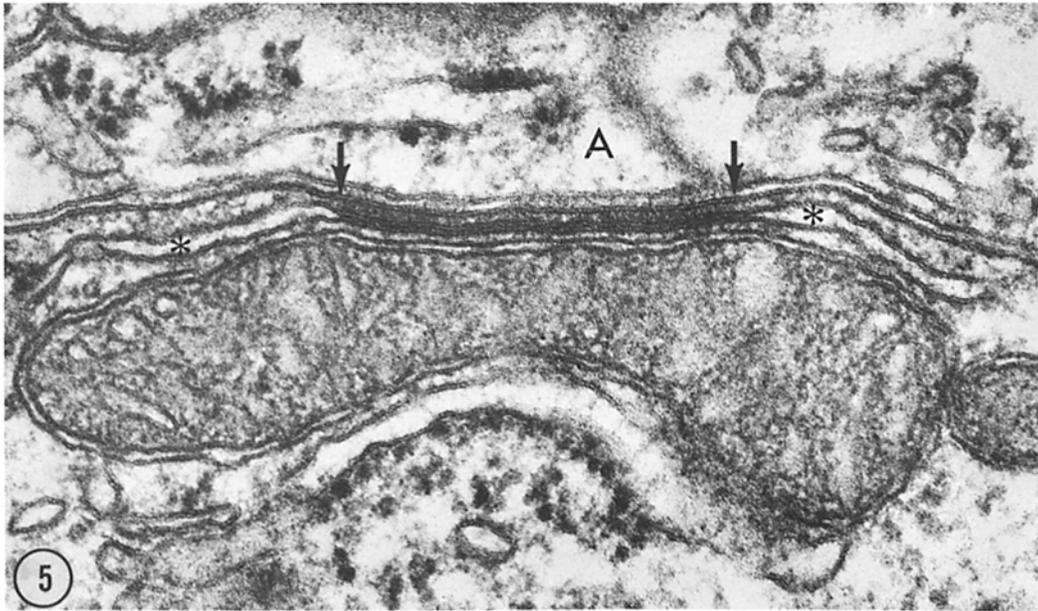
FIGURE 5   Subsurface cistern in Purkinje cell body. The lumen of the cistern (asterisks) is very narrow along the junction of the cistern with the plasma membrane of the Purkinje cell (between arrows). The plasmalemma lies parallel to the cisternal membrane along this contact, and the gap between them contains a fuzzy material. Mitochondria are frequently applied to these subsurface cisterns, and cisterns are frequent in regions of the Purkinje cell plasmalemma opposite astrocytes (A). × 90,000.

the general similarity of these junctions in a wide variety of muscles has led to the belief that they are sites of excitation-contraction coupling, whether they occur as triads, dyads, or with the surface membrane. The freeze-fracture technique has also demonstrated some differences between these junctions in different animals (15, 16), particularly differences in the density of large intramembrane particles in the T-system membrane at junctions with the sarcoplasmic reticulum. In vertebrate striated muscle, however, it has proven difficult to obtain images of fracture faces of the T-tubule and sarcoplasmic reticulum membranes at their junction (16). Possibly because of the sharp curvature of the transverse tubule membrane, the fracture plane tends to jump across the tubule rather than follow the tubule membrane.

The flat lamellae of hhe amphioxus body wall muscle are ideal for freeze-fracture studies of the surface-sarcoplasmic reticulum junction because fractures along the surface of the muscle are frequent, exposing large expanses of membrane on which junctional areas are unmistakable. In the amphioxus, as in all types of muscle junctions described so far, large particles (80–100 Å) characterize the surface membrane overlying junctions

with the sarcoplasmic reticulum. Fractures passing through the membranes of the sarcoplasmic reticulum cisterns were also common, but the fracture plane usually jumped out of the sarcoplasmic reticulum membrane just before passing through the true junctional region. Thus, images of the sarcoplasmic reticulum membrane unambiguously at the junction were rare, and our observations on this point scanty. Although structural studies of these junctions in amphioxus as in other muscles have shown clearly that they are not gap junctions (15), a type of structure which mediates long-termed electrotonic coupling between cells, the structural studies so far have not shown how these junctions might mediate excitation-contraction coupling.

Suggestions about the role of junctions between sarcoplasmic reticulum and the muscle surface membrane in excitation-contraction coupling have come from other approaches. In amphioxus muscle, current during the action potential is carried mainly by Na^+, but in the presence of tetrodotoxin or in the absence of Na^+ there is a measurable current carried by Ca^{++}. The Ca^{++} component of the action potential is blocked by La^{+++} which also blocks the muscle twitch (17, 18). When am-
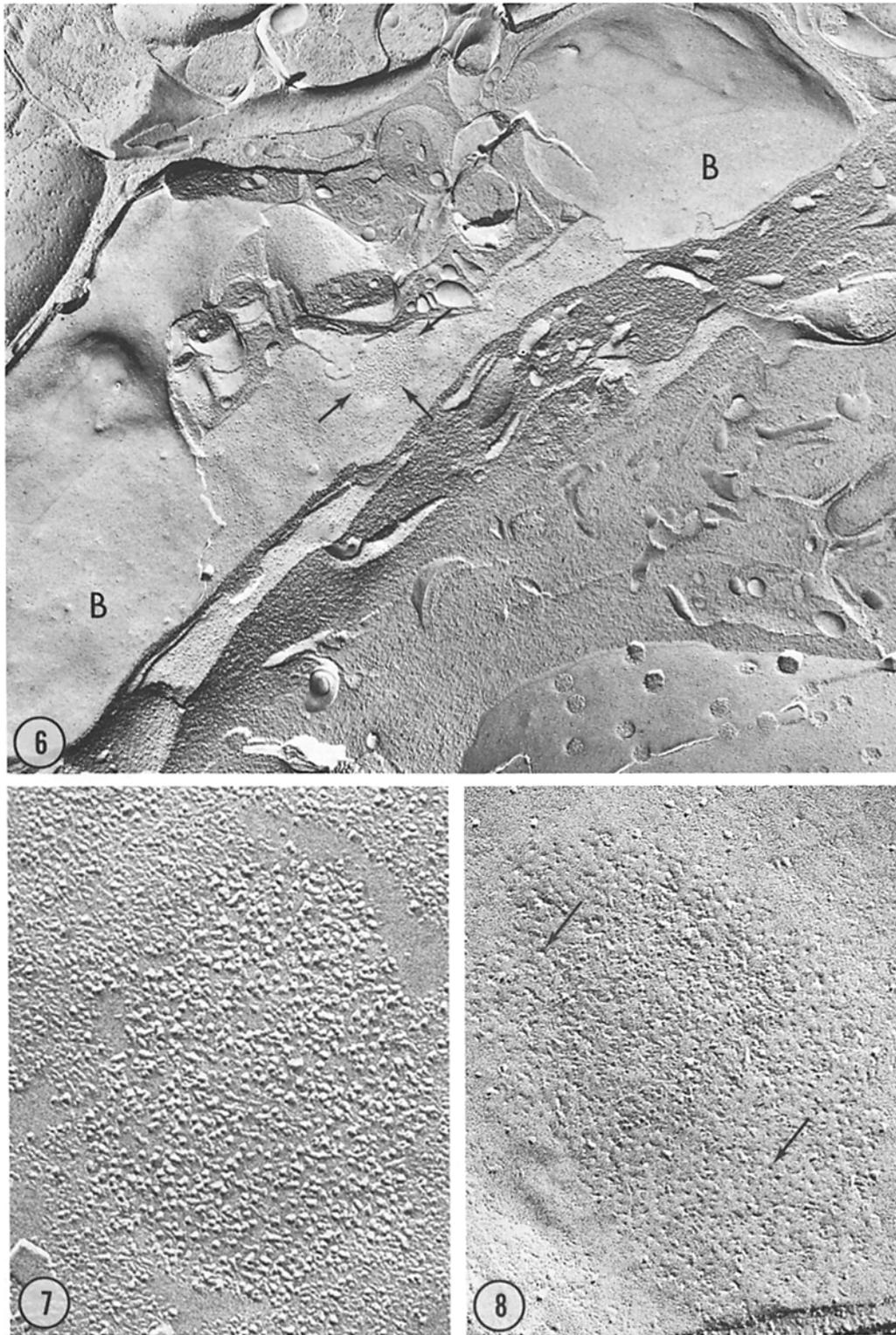
FIGURE 6 Freeze-fractured Purkinje cell body from mouse cerebellar cortex. Aggregates of particles (outlined by arrows) on the protoplasmic leaflet of the plasmalemma frequently occur on the surface of Purkinje cell bodies. External membrane leaflets of basket cell terminals are labeled (B). × 40,000.

FIGURES 7 and 8 Protoplasmic (Fig. 7) and external (Fig. 8) leaflets of the split plasma membrane of Purkinje cell bodies. Fig. 7 shows a characteristic aggregate of particles uniformly larger than the surrounding, dispersed intramembrane particles. Fig. 8 shows an aggregate of pits (arrows) on the external leaflet of the plasmalemma which could be complementary to particles such as those shown in Fig. 7. × 100,000.

FIGURE 9 Particle distribution on fracture faces of the Purkinje subsurface cistern membranes in the region of the junction. The point of view is outside the Purkinje cell body showing the protoplasmic leaflet (P) of the plasma membrane with its aggregate of large particles. The asterisk indicates a large step where the fracture jumps from the plasmalemma to the cisternal membrane exposing its luminal leaflet. Pits on the luminal leaflet of the cisternal membrane (large arrows) are the expected complementary image of particles. The presumably nonjunctional area to the left of the large arrows appears to have fewer pits. Thus, the plasmalemma and the outer membrane of the subsurface cistern are both marked by specialized particle arrays in the region of the cisternal-plasmalemmal contact. The small arrows indicate where the fracture jumps across the lumen of the cistern to expose the protoplasmic leaflet of the lower cistern membrane which also bears large particles. × 150,000.

phioxus muscle treated with La^{+++} at a concentration just sufficient to block the twitch is examined with the electron microscope, La^{+++} is found as particles scattered at low density over the surface membrane but is strikingly accumulated in the region of the junction between the surface and sarcoplasmic reticulum (17). This distribution of La^{+++} parallels, perhaps fortuitously, the distribution of large intramembrane particles in the surface membrane as revealed by the freeze-fracture technique. Although the identification of large intramembrane particles or the junctional area with sites of Ca^{++} entry is still speculative, it would be consistent with a mechanism suggested for excitation-contraction coupling (7, 35), in which Ca^{++} entry through the surface membrane at the junction would raise the cytoplasmic [Ca^{++}] in the vicinity to a level sufficient to trigger regenerative Ca^{++} release from the sarcoplasmic reticulum (9, 11). The physiological importance of this mechanism has been questioned for frog twitch muscle (1, 40, 43), but in frog twitch muscle fixed in Ca-

containing solutions electron-opaque particles, interpreted as indicative of Ca-binding sites, are localized either in the surface membrane or in the gap material at the triad junction (36). These sites must be distinct from sites of Ca stores in the muscle which should be predominantly within the sarcoplasmic reticulum (reviewed in reference 8). In any case, many other types of muscle do require external Ca^{++} for contraction in response to electrical stimulation (7, 8).

The main morphologic features of neuronal subsurface cistern junctions are very similar to those of junctions between sarcoplasmic reticulum cisterns and surface membrane in amphioxus muscle: (a) the intracellular components of the junction are part of the ER. (b) A part or all of the area of apposition between a subsurface cistern and the surface membrane participates in a specialized junction. (c) These junctions are characterized in thin sections by the parallel relationship between the surface and outer ER membranes as well as the fuzzy material in the roughly 20 nm gap
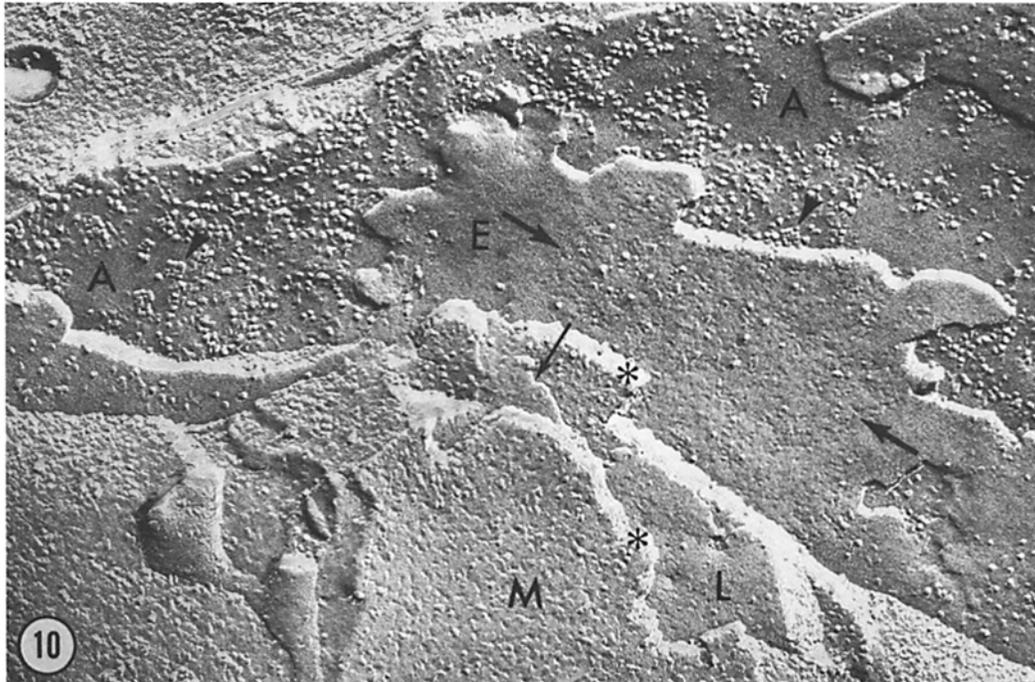
FIGURE 10 Subsurface cistern from point of view inside of Purkinje cell, exposing the external leaflet of its plasmalemma (E). A region of the plasmalemma which was in contact with the subsurface cistern is marked by many small pits (between large arrows, center) which are probably imprints of the large particles which mark the protoplasmic leaflet of the plasmalemma at the subsurface cistern. The plane of fracture jumps to the outer membrane of the cistern (upper asterisk), exposing its protoplasmic leaflet; jumps to the inner membrane of the cistern (small arrow, center) exposing its luminal leaflet (L); and finally jumps to the outer membrane of a mitochondrion (M, lower asterisk) exposing its protoplasmic leaflet. The mitochondrial membrane has linear incisures in its protoplasmic leaflet, which are imprints of the linear particles which characterize the other leaflet of its outer membrane. Apposed to the Purkinje cell plasmalemma around the subsurface cistern is the plasmalemma of an astrocyte (A). The exposed protoplasmic leaflet of its plasmalemma has numerous assemblies (arrowheads) and uniformly large particles, both characteristic of the astrocytic plasmalemma. Approx. × 90,000.

between them. This fuzz may appear as tufts across the gap or as a thin line about half way between the apposed membranes. (d) In freeze-fracture images, the protoplasmic leaflet of the surface membrane overlying subsurface cisterns, in both neurons and muscles, has an array of relatively large (80–100 Å) intramembrane particles. In neuronal subsurface cisterns, at the junction the membrane has a somewhat higher concentration of particles than the rest of the membrane of the cistern. For reasons mentioned above, insufficient data were obtained from amphioxus muscles to conclude that particles in the sarcoplasmic reticulum membrane were also concentrated at the junction, although it was clear that particles are found in this region as well as in the rest of the cisternal membrane.

The one major dissimilarity in structure between neuronal and muscle subsurface cisterns is the width of their lumens. The lumens of cisterns underlying junctions in the amphioxus muscle are wide whereas the lumens of subsurface cisterns in the cerebellar neurons shown in this study are very narrow if not occluded. However, the volume of subsurface cisterns may not be a definitive feature for several reasons. First, neuronal subsurface cisterns with different volumes have similar junctions with the surface membrane. For example, subsurface cisterns up to 600-μm wide occur in neurons of the rat cerebral cortex (38), and subsurface cisterns with open lumens also have clusters of intramembrane particles on the cytoplasmic half of overlying surface membrane in neurons of the frog sympathetic ganglion (6). On the other hand,

the volume of the amphioxus sarcoplasmic reticulum can be changed by various treatments. Soaking the muscle in physiological saline containing Ca^{++}, or adding 5 mM Ca^{++} to the fixative, causes the sarcoplasmic reticulum to swell (17), while soaking the muscle before fixation in saline, without added Ca^{++} but with 1 mM Ethyleneglycol-bis[β-aminoethyl·ether] N,N'-tetraacetic acid (EGTA), causes it to flatten. Soaking frog skeletal muscle in saline containing ruthenium red, a multivalent cation, results in collapse and occlusion of the lumen of portions of the sarcoplasmic reticulum (21). Thus, the volume of subsurface cisterns is probably not a definitive feature because it may reflect the physiological state of the cell, or interactions of its physiological state with the fixation and preparative conditions.

The extent of morphological similarity of the junctions between the surface membrane and subsurface cisterns in neurons and muscle includes many features of the distribution of the membrane particles revealed by the freeze-fracture technique. This, in combination with the fact that there is now some evidence that the ER of neurons as well as muscle cells may be capable of accumulating Ca^{++} (5, 19, 20, 28, 31), seems to justify consideration of the possibility that these junctions might have similar functions in these major types of excitable cells.

A mechanism in neurons for transmission of information about surface electrical activity to cytoplasmic systems could be of considerable importance. The balance between Ca^{++} release and Ca^{++} reuptake by endoplasmic reticulum might regulate a number of intracellular mechanisms, for example, glycogenolysis (32), actin-myosin interactions (22, 37), and packaging of neurotransmitters (3). The intracellular Ca^{++} concentration also can control the membrane permeability to other ions in at least some neurons. An increase in the membrane permeability to potassium is produced by increased intracellular Ca^{++} in neurons of snail (29), Aplysia (30), and mammalian spinal motor neurons (10, 24). The increase in potassium permeability results in hyperpolarization of these cells. Release of Ca^{++} from the endoplasmic reticulum, for example, with repetitive firing or prolonged stimulation could result in an increase of K^+ conductance, such as that seen in some types of poststimulus hyperpolarization (4). In fact, subsurface cisterns are located beneath the postsynaptic membrane at certain synapses (reference 2 and reviewed in reference 33). In no case has the

mechanism of action of such synapses been worked out in detail, but we would predict that they are designed to release Ca into the cytoplasm with whatever further effects this might produce. Thus, there now are many examples, in neurons as well as muscle cells, where excitation coupling to intracellular processes or to modulation of surface membrane properties may depend on Ca^{++} release from the endoplasmic reticulum.

REFERENCES

1. ARMSTRONG, C. M., F. M. BENZANILLA, and P. HOROWICZ. 1972. Twitches in the presence of ethyleneglycolbis(β-aminoethyl ether)-N-N'tetraacetic acid. Biochim. Biophys. Acta. 267:605-608.

2. BODIAN, D. 1972. Synaptic diversity and characterization by electron microscopy. In Structure and Function of Synapses. G. D. Pappas and D. P. Purpura, editors. Raven Press, New York. 45-65.

3. BOROWITZ, J. L., K. FUWA, and N. WEINER. 1965. Distribution of metals and catecholamines in bovine adrenal medulla sub-cellular fractions. Nature (Lond.). 205:42-43.

4. BRODWICK, M. S., and D. JUNGE. 1972. Post-stimulus hyperpolarization and slow potassium conductance increase in Aplysia giant neurone. J. Physiol. 223:549-570.

5. DIAMOND, I., and A. L. GOLDBERG. 1971. Uptake and release of ^{45}Ca by brain microsomes, synaptosomes and synaptic vesicles. J. Neurochem. 18:1419-1431.

6. DICKINSON, A. H., and T. S. REESE. 1974. Interactions of synaptic vesicles with the plasmalemma in frog sympathetic ganglia. J. Cell Biol. 63(2, Pt. 2):83 a. Abstr.

7. EBASHI, S., and M. ENDO. 1968. Calcium ion and muscle contraction. Prog. Biophys. Mol. Biol. 18:125-183.

8. EBASHI, S., M. ENDO, and I. OHTSUKI. 1969. Control of muscle contraction. Q. Rev. Biophys. 2:351-384.

9. ENDO, M., M. TANAKA, and Y. OGAWA. 1970. Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibres. Nature (Lond.). 228:34-36.

10. FELTZ, A., K. KRNJEVIC, and A. LISIEWICZ. 1972. Intracellular free Ca^{2+} and membrane properties of motoneurons. Nat. New Biol. 237:179-181.

11. FORD, L. E., and R. J. PODOLSKY. 1970. Regenerative calcium release within muscle cells. Science

(Wash. D. C.). **167**:58-59.

12. Franzini-Armstrong, C. 1964. Fine structure of sarcoplasmic reticulum and transverse tubular system in muscle fibers. *Fed. Proc.* **23**:887-895.

13. Franzini-Armstrong, C. 1970. Studies of the triad. I. Structure of the junction in frog twitch fibres. *J. Cell Biol.* **47**:488-499.

14. Franzini-Armstrong, C. 1973. Studies of the triad. IV. Structure of the junction in frog slow fibers. *J. Cell Biol.* **56**:120-128.

15. Franzini-Armstrong, C. 1974. Freeze-fracture of skeletal muscle from the tarantula spider. Structural differentiations of sarcoplasmic reticulum and transverse tubular system membranes. *J. Cell Biol.* **61**:501-513.

16. Franzini-Armstrong, C. 1975. Membrane particles and transmission at the triad. *Fed. Proc.* **34**:1382-1389.

17. Hagiwara, S., M. Henkart, and Y. Kidokoro. 1971. Excitation-contraction coupling in amphioxus muscle cells. *J. Physiol. (Lond.).* **219**:233-251.

18. Hagiwara, S., and Y. Kidokoro. 1971. Na and Ca components of action potential in amphioxus muscle cells. *J. Physiol. (Lond.).* **219**:217-232.

19. Henkart, M. 1972. Structure and function of the endoplasmic reticulum in the squid giant axon. Proceedings of the Second Annual Meeting of the Society for Neuroscience. 103. Abstr.

20. Henkart, M. 1975. The endoplasmic reticulum of neurons as a calcium sequestering and releasing system: morphological evidence. *Biophys. J.* **15**:267 a. (Abstr.).

21. Howell, J. N. 1974. Intracellular binding of ruthenium red in frog skeletal muscle. *J. Cell Biol.* **62**:242-245.

22. Ishikawa, H., R. Bischoff, and H. Holtzer. 1969. Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. *J. Cell Biol.* **43**:312-328.

23. Kelly, D. E. 1969. The fine structure of skeletal muscle triad junctions. *J. Ultrastruct. Res.* **29**:37-49.

24. Krnjevic, K., and A. Lisiewicz. 1972. Injections of calcium ions into spinal motoneurons. *J. Physiol.* **225**:363-390.

25. Landis, D. M. D., M. Henkart, and T. S. Reese. 1973. Similar arrays of plasma membrane particles at subsurface cisterns in striated muscle and neurons. *J. Cell Biol.* **59**(2, Pt. 2):184 a. (Abstr.).

26. Landis, D. M. D., and T. S. Reese. 1974. Arrays of particles in freeze-fractured astrocytic membranes. *J. Cell Biol.* **60**:316-320.

27. LeBeux, Y. J. 1972. Subsurface cisterns and lamellar bodies: particular forms of the endplasmic reticulum in the neurons. *Z. Zellforsch. Mikrosk. Anat.* **133**:327-352.

28. Lieberman, E. M., R. F. Palmer, and G. H. Collins. 1967. Calcium ion uptake by crustacean peripheral nerve subcellular particles. *Exp. Cell Res.* **46**:412-418.

29. Meech, R. W. 1974. The sensitivity of *Helix aspersa* neurons to injected calcium ions. *J. Physiol.* **237**:259-277.

30. Meech, R. W., and F. Strumwasser. 1970. Intracellular calcium injection activates potassium conductance in *Aplysia* nerve cells. *Fed. Proc.* **29**:834.

31. Otsuka, M., I. Otsuki, and S. Ebashi. 1965. ATP-dependent Ca-binding of brain microsomes. *J. Biochem. (Tokyo).* **58**:188-190.

32. Ozawa, E. 1973. Activation of phosphorylase kinase from brain by small amounts of calcium ion. *J. Neurochem.* **20**:1487-1488.

33. Pappas, G. D., and S. G. Waxman. 1972. Synaptic fine structure-morphological correlates of chemical and electrotonic transmission. In Structure and Function of Synapses. G. D. Pappas and D. P. Purpura, editors. Raven Press, New York. 1-43.

34. Peachey, L. D. 1964. Transverse tubules in excitation-contraction coupling. *Fed. Proc.* **24**:1124-1134.

35. Podolsky, R. J. 1975. Muscle activation: the current status. *Fed. Proc.* **34**:1374-1378.

36. Politoff, A. L., S. Rose, and G. D. Pappas. 1974. The calcium binding sites of synaptic vesicles of the frog sartorius neuromuscular junction. *J. Cell Biol.* **61**:818-823.

37. Puszkin, S., S. Berl, E. Puszkin, and D. D. Clarke. 1968. Actomyosin-like protein isolated from mammalian brain. *Science (Wash. D. C.).* **161**:170-171.

38. Rosenbluth, J. 1962. Subsurface cisterns and their relationship to the neuronal plasma membrane. *J. Cell Biol.* **13**:405-422.

39. Sandow, A. 1965. Excitation-contraction coupling in skeletal muscle. *Pharmacol. Rev.* **17**:265-320.

40. Sandow, A., M. K. D. Pagala, and E. C. Sphicas. 1974. "Zero" Ca effects on excitation-contraction coupling (ECC). *Fed. Proc.* **33**:1259.

41. Siegesmund, K. A. 1968. The fine structure of subsurface cisterns. *Anat. Rec.* **162**:187-196.

42. Takahashi, K., and R. L. Wood. 1970. Subsurface cisterns in the Purkinje cells of cerebellum of Syrian hamster. *Z. Zellforsch. Mikrosk. Anat.* **110**:311-320.

43. Thorens, S., and M. Endo. 1975. Calcium-induced calcium release and "depolarization"-induced calcium release: their physiological significance. *Proc. Jpn. Acad.* **51**:473-478.