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CONTRACTIONS OF THE SQUID STELLATE GANGLION

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Summary

Optical recording methods were used to characterize the intrinsic movements of squid stellate ganglia. Ganglionic contractions were rhythmic and occurred at a mean frequency of $8\,min^{-1}$. Contractions were eliminated when Na^+ in the external saline were replaced by Tris, but were only slowed when Na^+ was replaced by sucrose. This suggests that Na^+ plays some role in generating the contractions, but the complete abolition produced by Tris-containing saline may be due to a secondary pharmacological action of this ion. The Na^+ channel blocker, tetrodotoxin, had no effect on contractions. Contractions were eliminated by removal of external Ca^{2+}, by treatment with the inorganic Ca^{2+} channel blockers Mn^{2+} and Cd^{2+}, or by treatment with the organic dihydropyridine Ca^{2+} channel blockers nitrendipine and nimodipine. Thus, extracellular Ca^{2+} plays an important role in generating the contractions. Because dihydropyridines eliminate contractions, but not synaptic transmission, they offer a means of studying transmission at the giant synapse of the stellate ganglion without having to contend with ganglionic movement. Electron microscopy of stellate ganglia revealed the presence of two types of cell that contained the organized arrays of cytoskeletal elements usually associated with contractile cells. One type was a pericyte that surrounded blood vessels within the stellate ganglion. The second type was distributed throughout the ganglion and resembled a smooth muscle cell. Either of these cell types might generate ganglionic contractions.

Introduction

Some types of nervous tissues have been reported to move. For example, optical studies of the central nervous system of vertebrates have shown large, slow changes in light transmission that are caused by brain movements (Grinvald *et al.*

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1984; Orbach *et al.* 1985). These movements appear to be largely related to the activity of the circulatory and/or respiratory systems and may not reflect intrinsic contractile activity of the brain. The nervous systems of certain invertebrates have also been found to move (e.g. Alexandrowicz, 1963; Mirolli and Gorman, 1968; Grinvald *et al.* 1981; London *et al.* 1987). These movements can be detected in isolated nervous tissue and thus appear to be due to the intrinsic contractility of these nervous systems.

In this paper we characterize the intrinsic movements of the squid stellate ganglion, an important model system for the study of synaptic function (Llinas, 1982). We describe some of the basic features of the contractility of this ganglion and examine the ionic basis of these contractions. We also identify treatments that block contractions without altering transmission at the giant synapse of this ganglion. Because these contractions have made it difficult to study the giant synapse, this information will facilitate future studies of synaptic transmission. We have also found within the ganglion two types of cells that exhibit the structural specializations expected of contractile cells. These cells may be responsible for producing the ganglionic contractions. Preliminary accounts of this work have appeared in abstracts (Sanchez and Augustine, 1988; Nuño *et al.* 1988).

Materials and methods

Ganglion isolation

Stellate ganglia of the squid, *Loligo pealei* Lesueur, were isolated as described in Augustine and Eckert (1984). These ganglia were carefully dissected to remove all adhering connective tissue, blood vessels and contractile epithelium. Isolated ganglia were pinned to a layer of Sylgard coating the bottom of a recording chamber, constructed from a 22 mm × 40 mm coverslip, and stretched to approximately their *in vivo* length. Experiments were performed at room temperature, which ranged from 21 to 25 °C.

Recording procedures

The recording method employed takes advantage of the fact that substantial changes in light transmission occur as tissue moves (e.g. Baylor *et al.* 1982). Our method was based on the use of a photodiode to measure such changes from moving ganglia and was derived from the techniques used by Kupferman *et al.* (1974) to measure contractions of the *Aplysia* gill.

Our recording apparatus was designed with the intention of using routinely available commercial equipment as much as possible. The arrangement used is shown in Fig. 1. The recording chamber, containing a ganglion, was mounted on the stage of a dissecting microscope. The ganglion was transilluminated with an ordinary microscope illuminator (Bausch & Lomb, model 31–32–42), although the use of a light source with a better-regulated power supply (e.g. Smith, 1986) would undoubtedly have yielded much lower noise levels. Light transmitted through the ganglion was collected with a 2-mm diameter plastic fiber optic light

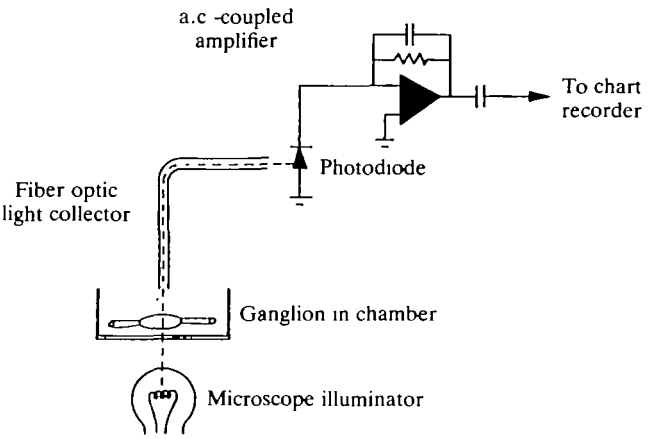

Fig. 1. Experimental arrangement for optical recording of ganglion movements. Changes in light transmission through the ganglion were measured with a photodiode and the resultant signals stored on a chart recorder.

pipe. To limit the size of the area sampled, this collector was tapered to a final diameter of approximately 100 μm by heating.

The fiber optic light pipe transmitted the collected light to a photodiode (type UV-040B, EG&G ElectroOptics, Salem, MA). The photocurrent flowing through this diode was measured with an a.c.-coupled current-to-voltage converter and displayed on a chart recorder. The feedback resistor and capacitor on the current amplifier (AD 545) were 100 MΩ and 3 nF, respectively, and the a.c.-coupling capacitor between the amplifier and recorder was 50 nF. These values were selected empirically to optimize the signal-to-noise ratio of the system while providing minimal distortion to the recorded signals, although the a.c.-coupling produced some distortion of the very slowest signals considered here. The effective frequency response of the system (3 dB attenuation) was approximately 0.01–4 Hz (or 1–200 min^{-1}).

Physiological solutions

Our usual squid saline consisted of 466 mmol l^{-1} NaCl, 54 mmol l^{-1} $MgCl_2$, 11 mmol l^{-1} $CaCl_2$, 10 mmol l^{-1} KCl, 3 mmol l^{-1} $NaHCO_3$ and 10 mmol l^{-1} Na–Hepes, pH 7.2. The osmotic pressure of different batches of this saline ranged from 1070 to 1090 mosmol kg^{-1}. Tris–Cl (505 mmol l^{-1}) and sucrose (670 mmol l^{-1}) were used as iso-osmotic substitutes for NaCl in Na^+-free saline. $MgCl_2$ (65 mmol l^{-1} total) was used as a replacement for $CaCl_2$ in Ca^{2+}-free saline. $MnCl_2$ and $CdCl_2$ were added to the normal saline without osmotic compensation. All the reagents used in these solutions, as well as tetrodotoxin, were obtained from Sigma Chemical Co.

The dihydropyridines, nitrendipine and nimodipine, were obtained from Miles Laboratories (West Haven, CT). These compounds were dissolved in dimethylsulfoxide (DMSO) to prepare stock solutions of 20 mmol l^{-1}. Stock

solutions were frozen and stored in the dark until use. The stock solutions were defrosted immediately before use and dissolved in saline by vigorous agitation. Before ganglia were exposed to the dihydropyridine-containing saline they were treated with control salines that contained identical concentrations of DMSO (usually 0.1 % or less) but no dihydropyridines. This treatment had no obvious effect on the contractions.

Electron microscopy

Stellate ganglia were fixed in 2 % glutaraldehyde in 0.1 mol l^{-1} cacodylate buffer containing 0.8 mol l^{-1} sucrose and 3 % DMSO. This fixative and all solutions used through the osmium post-fixation step had a pH of 7.4 and an osmolality of 1200 mosmol kg^{-1}. After 2 h of fixation at room temperature, the tissue was fixed for a further 12 h at 4 °C. Following a rinse in 0.1 mol l^{-1} cacodylate buffer, the tissue was post-fixed in 1 % OsO_4 in 0.1 mol l^{-1} cacodylate buffer containing 0.8 % potassium ferricyanide. After rinsing in distilled water, the ganglia were stained *en bloc* in 2 % uranyl acetate for 2 h, then dehydrated in an ethanol series and propylene oxide. Dehydrated ganglia were flat-embedded in an Epon–Araldite mixture and thin-sectioned on a Reichert ultramicrotome. The sections were post-stained in uranyl acetate and lead citrate and examined in a JEOL 100 CXII electron microscope operated at an accelerating voltage of 60 kV.

Results

Characteristics of ganglionic movements

Most isolated stellate ganglia exhibited spontaneous movements. These movements were sometimes sufficiently large to be observed through the microscope, but they were detected with higher sensitivity by using the optical recording technique illustrated in Fig. 1. When the contractions could be visually detected they appeared to coincide temporally with signals in the optical recordings, suggesting that the optical method was reliably reporting ganglionic movements rather that some other intrinsic optical signal (e.g. Cohen *et al.* 1968).

Examples of optical recordings of ganglionic contractions are shown in Fig. 2. Contraction signals recorded from a single position in a given ganglion were usually very regular in their amplitude and frequency. An example of this type of activity is shown at the top of Fig. 2. Such activity was observed in more than 95 % of the ganglia bathed in normal squid saline. The variable-amplitude signal shown in the middle trace was seen in only one ganglion and the variable-frequency, 'bursting' type of activity was observed in two other ganglia.

The amplitude of the optical signals associated with ganglionic movement depended upon the location of the fiber optic light collector. As expected, the amplitude of these signals was largest at boundaries between regions with different light-transmission properties. For example, in the experiment shown in Fig. 3, the optical signals were largest at position A, a boundary between the ganglion and the saline, and at position F, a boundary between the opaque ganglionic neuropil and

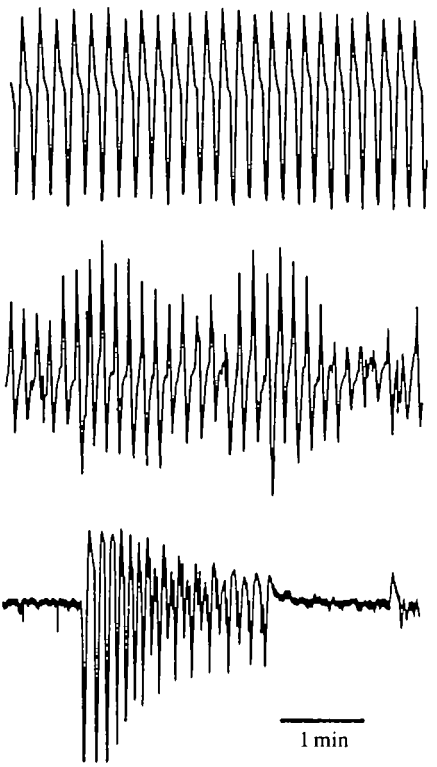

Fig. 2. Examples of ganglion contraction signals. Vertical scaling of signals is arbitrary in this and subsequent figures.

a relatively transparent giant axon. Recordings over portions of the ganglion with more uniform optical properties (e.g. positions B–E) yielded smaller signals as the ganglion moved under the light collector. This position-sensitivity made it difficult to quantify the magnitude of the signals, so we have not attempted to calibrate their amplitude. As an order-of-magnitude calibration, these signals represent 0.1–0.01 % changes in light transmission.

In contrast to their amplitude, the frequency of contractions in a given ganglion was independent of the position of the light collector. This is illustrated in Fig. 3 by the temporal alignment of the peaks of the optical signals recorded at various positions. Since this permitted reliable measurements of contraction frequency, we have quantified the effect of experimental manipulations on the rate of contractions. Pooling data on the basal contraction frequency of 61 ganglia bathed in normal saline revealed that these frequencies were more or less normally distributed, with a mean of $8\,min^{-1}$ (Fig. 4).

Ionic basis of ganglion contractions

We next performed a number of experiments to elucidate the ionic mechanisms responsible for these spontaneous contractions. In particular, we examined the

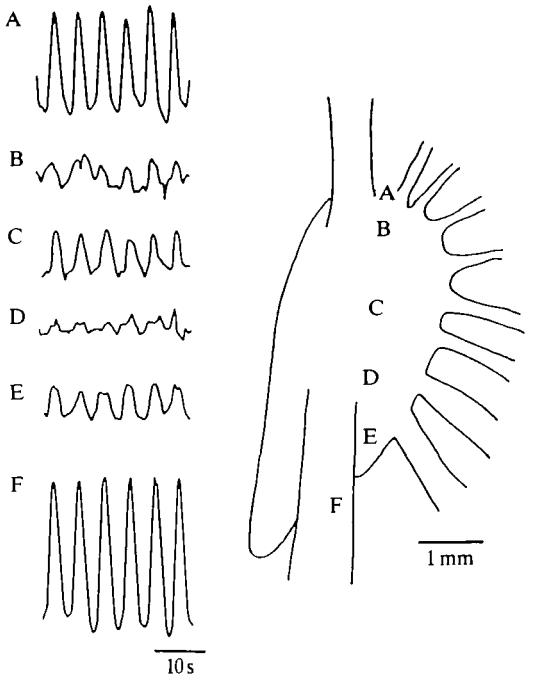

Fig. 3. Movement signals vary according to the position within the ganglion. (A–F) Left, recordings obtained at various positions in a single ganglion; right, schematic diagram of this ganglion. The letters indicate the position of the light collector when the corresponding traces, shown on the left, were recorded.

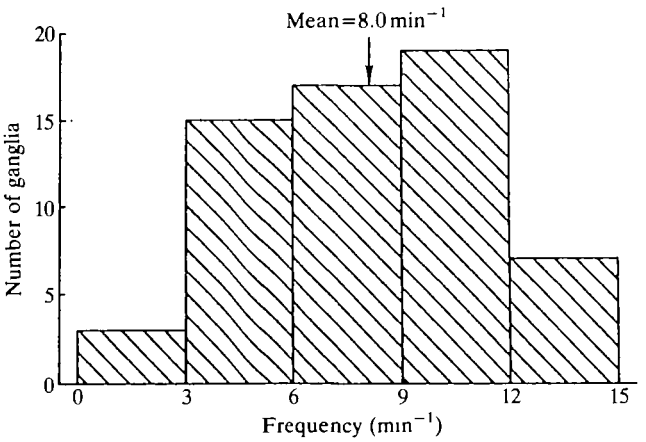

Fig. 4. Distribution of contraction frequencies in a total of 61 isolated ganglion preparations.

role of Na^+ and Ca^{2+} in the generation of contractions, since these two ions are almost universally involved in initiating electrical activity in excitable cells (Hille, 1984).

Role of Na^+

We first addressed the role of Na^+ by removing it from the external medium. Contractions were eliminated when Tris ions, which do not generally permeate through Na^+ channels (Hille, 1984), were used as a substitute for Na^+ in the squid saline (Fig. 5A). Na^+-free, Tris–substituted [$0Na^+$(Tris)] saline produced a rapid decrease in both the amplitude and frequency of contractions, although during the onset of its effect contraction frequency was reduced to a greater extent than was amplitude. In 12 experiments the frequency of contractions was reduced to 0.02 $\pm$0.02 ($\pm$S.E.M.) of the control value recorded prior to exposure to $0Na^+$(Tris) saline. This elimination of contractions was rapidly reversed when normal saline was returned to the recording chamber.

In contrast, contractions persisted when sucrose was used as a substitute for Na^+ (Fig. 5B). During prolonged exposure to this saline [$0Na^+$(sucrose)] the frequency of contractions usually declined (mean reduction to 0.34$\pm$0.06 of control in 18 experiments), but in 15 of 18 experiments contractions could be detected after exposing ganglia to $0Na^+$(sucrose) saline for as long as 45 min. The amplitude of the contractions was also somewhat reduced by prolonged exposure to $0Na^+$(sucrose) saline, decreasing by roughly the same extent as the frequency.

This distinction between the actions of these two Na^+-free salines was most evident in experiments in which single preparations were exposed to both. An example of such an experiment is shown in Fig. 5C. In this experiment, exposure to $0Na^+$(Tris) saline produced a rapid elimination of contractions. Subsequent exposure to $0Na^+$(sucrose) saline restored contractions, albeit at a lower frequency. This experiment shows that contractions could be sustained in $0Na^+$(sucrose) saline even after Na^+ had previously been replaced by Tris, decreasing the likelihood that the contractions recorded in $0Na^+$(sucrose) were caused by a slow removal of Na^+ during exposure to $0Na^+$ saline. Similar results were seen in five replicates of this experiment and also in six related experiments in which treatment with $0Na^+$(Tris) saline followed exposure to $0Na^+$(sucrose) saline. Thus, contractions were sustained in Na^+-free saline if sucrose, but not Tris, was used as a Na^+ substitute.

The reduction of contraction frequency produced by $0Na^+$(sucrose) saline indicates that Na^+ plays some role in determining the rate of spontaneous contractions. However, because the contractions were not completely eliminated by this treatment we conclude that Na^+ alone is not responsible for initiating contractions. The rapid abolition of contractions observed in $0Na^+$(Tris) saline may have been caused by a secondary pharmacological action of the Tris (e.g. Gillespie and McKnight, 1976).

To examine further the role of Na^+ channels in the generation of contractions, we examined the action of the Na^+ channel blocker tetrodotoxin (TTX) on the

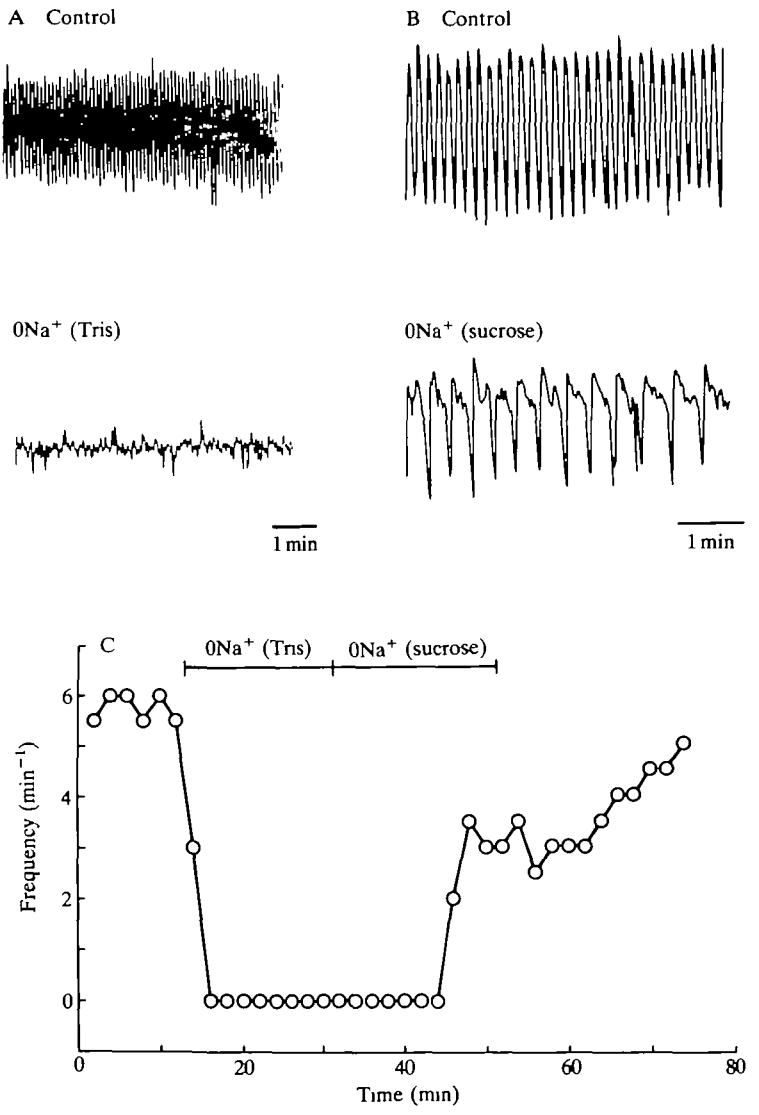

Fig. 5. Role of Na^+ in ganglion contractions. (A) Recordings in normal saline (Control) and in saline in which Na^+ was replaced by an equimolar amount of Tris [$0Na^+$(Tris)]. (B) Recordings in normal saline (Control) and saline in which Na^+ was replaced by iso-osmotic sucrose [$0Na^+$(sucrose)]. (C) Time course of ganglion contractions during sequential exposure to Na^+-free saline containing Tris or sucrose as a substitute for Na^+.

contractions. At a concentration of 1 μmol l^{-1}, TTX had no significant effect upon contraction amplitude or frequency (mean frequency 0.98±0.04 of control; N=4), even after 30 min of exposure. This concentration of TTX is capable of completely eliminating currents flowing through voltage-gated Na^+ channels of squid neurons

and of many other cells (Kao, 1986). We therefore conclude that Na^+ is not influencing contraction frequency by permeating through TTX-sensitive channels.

Role of Ca^{2+}

We next tested whether Ca^{2+} might be involved in generation of the ganglionic contractions by examining the effects of Ca^{2+} removal and of pharmacological agents that affect Ca^{2+} channels.

Ca^{2+} was removed from the external saline by replacement with Mg^{2+}. Treatment of ganglia with this Ca^{2+}-free saline eliminated contractions in each of 11 experiments (Fig. 6A). This reduction in contraction frequency was prompt and reversible, although Ca^{2+} restoration often produced a transient overshoot in contraction frequency (Fig. 6B). Exposure to saline with Ca^{2+} concentrations between 0 and 11 mmol l^{-1} produced intermediate changes in contraction frequency (not shown) and was often accompanied by an enhancement in contraction amplitude over that recorded in saline containing 11 mmol l^{-1} Ca^{2+}.

Treatment of ganglia with inorganic Ca^{2+} channel blockers also eliminated contractions. In these experiments the Ca^{2+} channel blocking ions Mn^{2+} and Cd^{2+} (Hagiwara and Byerly, 1981) were added to saline containing the normal concentration of Ca^{2+} (11 mmol l^{-1}). An example of the effect of Mn^{2+} is shown in Fig. 7. Mn^{2+} completely blocked the contractions (mean frequency=0.0 of control, N=8) at concentrations as low as 6.3 mmol l^{-1}, whereas Cd^{2+} was more potent, eliminating contractions at concentrations of 2 mmol l^{-1}. The mean frequency of contractions in 2 mmol l^{-1} Cd^{2+} was 0.05±0.05 of control values recorded prior to Cd^{2+} treatment. Both these ions eliminated contractions within

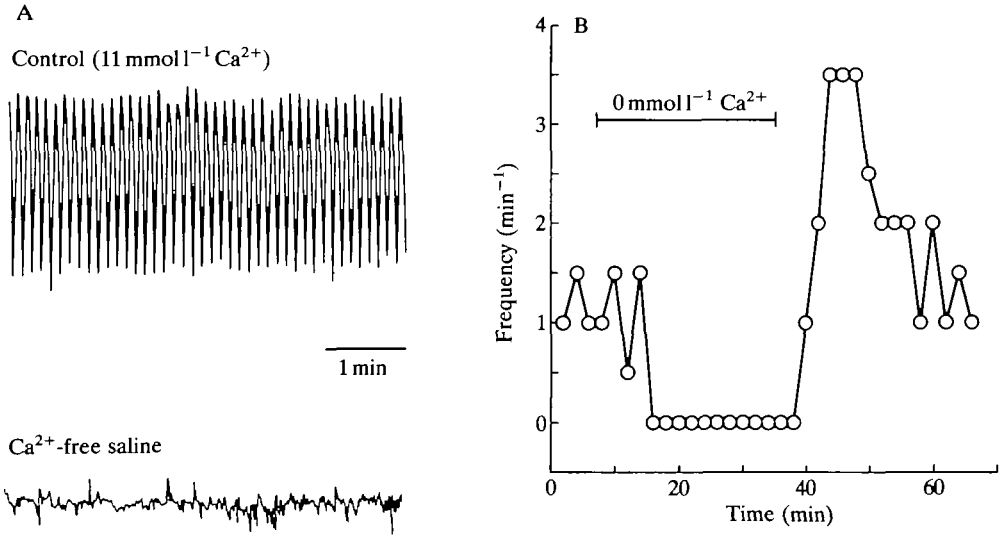

Fig. 6. Ca^{2+} removal eliminates ganglion contractions. (A) Recordings of ganglion movement in saline containing 11 mmol l^{-1} Ca^{2+} (Control) or no Ca^{2+} (Ca^{2+}-free saline). (B) Time course of effects of exposure to Ca^{2+}-free saline on contraction frequency.

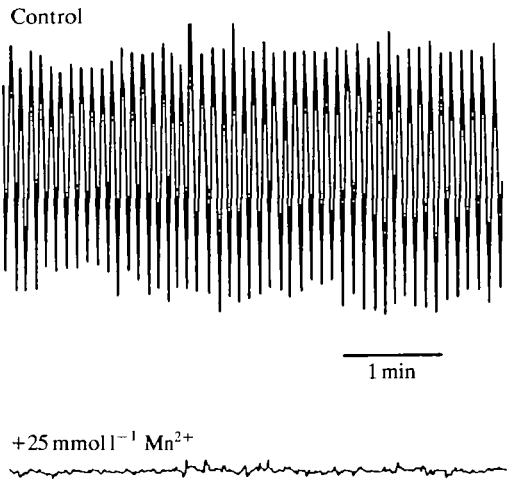

Fig. 7. Contractions present in normal saline (Control) were eliminated by addition of Mn^{2+} to the saline.

10 min of their addition to the recording chamber and their actions were irreversible. These blockers appeared to have little direct effect on contraction amplitude because contractions recorded during the onset of block were similar in amplitude to those recorded before adding blockers.

We also examined the action of the organic dihydropyridine (DHP) Ca^{2+} channel antagonists, nitrendipine and nimodipine (Hess *et al.* 1984). Both of these agents were capable of eliminating ganglionic movements, although their effects were variable. In five of eight experiments, 10 μmol l^{-1} nitrendipine eliminated contractions. An example of such an effect of nitrendipine is shown in Fig. 8A. The elimination of contractions by nitrendipine was usually due to a gradual reduction in contraction amplitude, although in two experiments contraction frequency also declined gradually (e.g. Fig. 8B). These effects were not reversed even after more than 30 min of exposure to drug-free saline. In one experiment, 20 μmol l^{-1} nimodipine produced a gradual reduction in contraction amplitude that eliminated the contractions within 40 min. In four experiments ganglia were treated with very high (nominally 200 μmol l^{-1}) concentrations of nimodipine; in these experiments ganglionic contractions were eliminated within 5 min of exposure to the drug. Although this acceleration in the rate of action of nimodipine might have been caused by a secondary, non-specific effect of the drug, it could also suggest that lower concentrations of DHPs act more slowly because these drugs only slowly reach the necessary concentrations at their sites of action. Thus, DHP antagonists were much more potent than the inorganic Ca^{2+} channel blockers, although their actions were slower, more variable and predominantly expressed as changes in contraction amplitude.

In conclusion, both Ca^{2+} removal and treatment with Ca^{2+} channel blockers eliminated ganglion contractions. These results indicate that extracellular Ca^{2+}

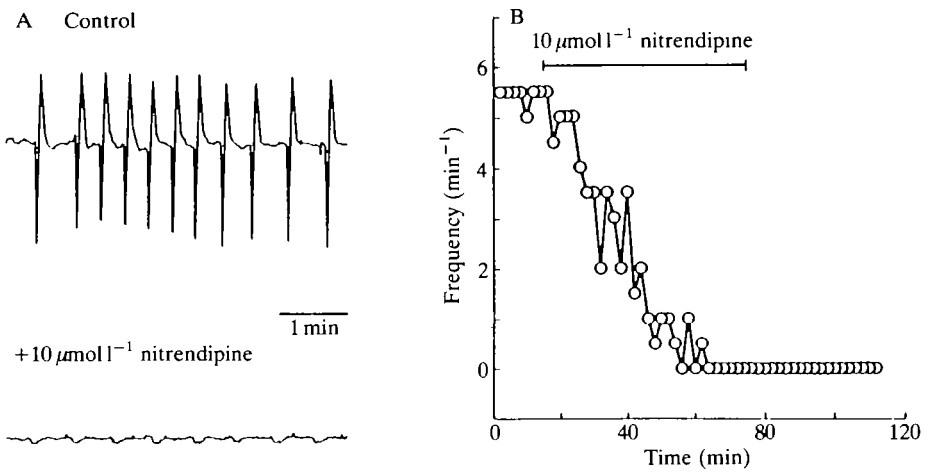

Fig. 8. The dihydropyridine antagonist compound, nitrendipine, eliminates contractions. (A) Recordings of ganglion movement in saline without (Control) or with $10\,\mu mol^{-1}$ nitrendipine. (B) Time course of effects of exposure to nitrendipine on contraction frequency.

plays an important role in generating the ganglion contractions. The results with Ca^{2+} channel blockers further suggest that influx of Ca^{2+} through Ca^{2+} channels is at least part of the means by which Ca^{2+} is involved in contraction.

Contractile elements within the stellate ganglion

Electron microscopy was used to identify contractile cells within the stellate ganglion. Cells which resembled the smooth muscle cells of other molluscs (Nicaise and Amsellem, 1983; Chantler, 1983) were found throughout the ganglion (Fig. 9A,B). These smooth muscle cells were embedded in a layer of collagen fibers and were most abundant near the surface of the ganglion. When sectioned perpendicular to the long (anterior–posterior) axis of the ganglion, the cells were oval or spindle-shaped. Occasionally they had long processes that extended over several micrometers in a single section. We saw no indication of junctions connecting these cells to each other or to other cells within the ganglion.

The cytoplasm of the smooth muscle cells was more electron-dense than that of the surrounding neurons. The cytoplasm typically contained membrane-bound cisternae just beneath the plasma membrane (Fig. 10). Occasionally the region beneath the plasma membrane also contained an electron-dense zone (Fig. 10) that may correspond to the attachment plate or dense body found in some types of molluscan smooth muscle cells (Nicaise and Amsellem, 1983). Mitochondria were present at a low density in these cells, and a nucleus or Golgi apparatus was rarely seen (Fig. 9B).

The most distinctive features of the smooth muscle cells were arrays of small, electron-dense structures that appeared to be contractile filaments cut in cross-section (Fig. 9B, inset). One type of filament was irregular in shape, approxi-

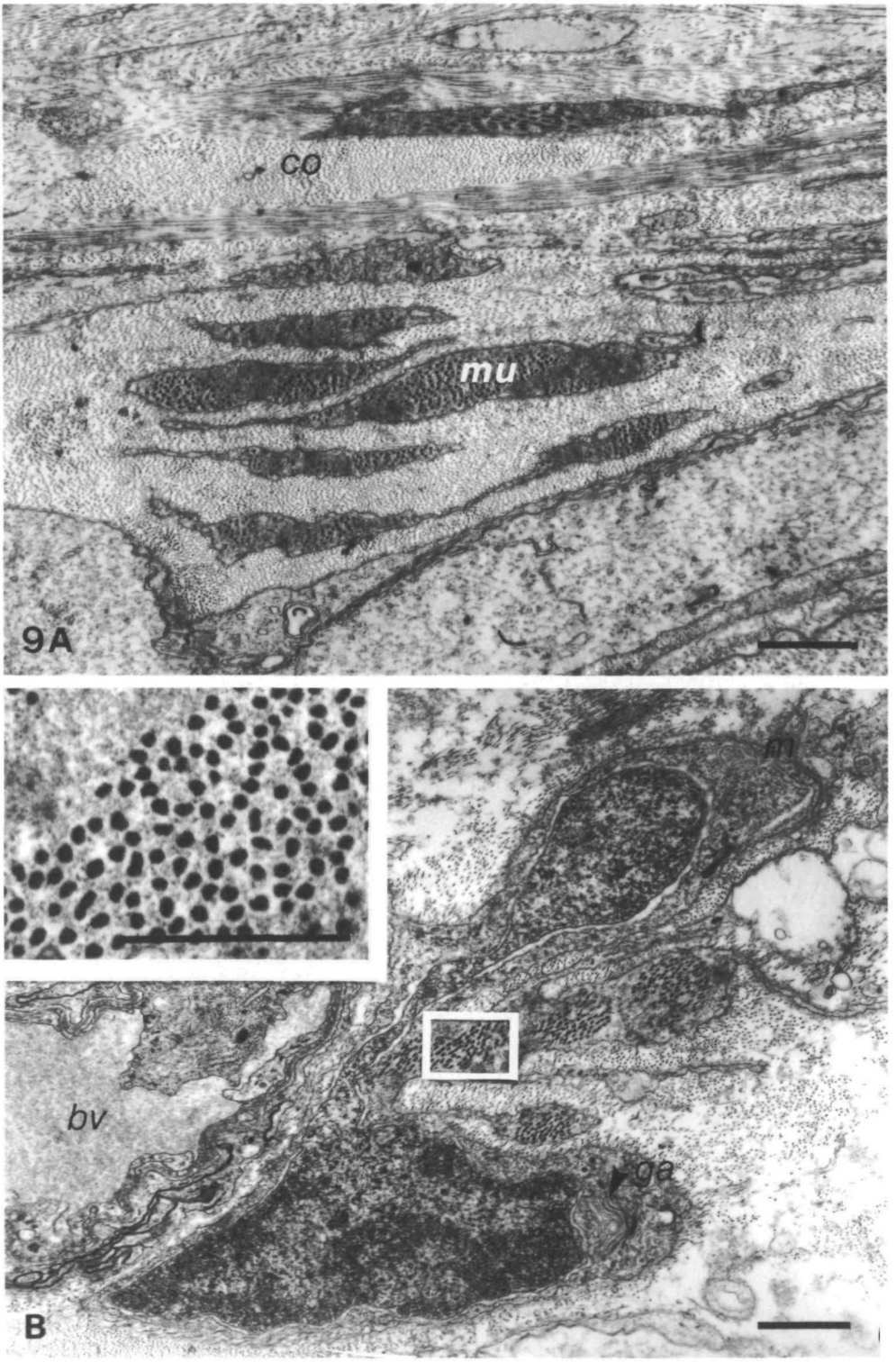
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Fig. 9. Electron micrographs of smooth muscle cells of the squid stellate ganglion. (A) Image of several smooth muscle cells (*mu*) in a layer of collagen fibers (*co*). Several of the muscle cells have long cytoplasmic processes. A neuronal axon is visible in the lower right region of the micrograph. (B) Morphology of a single smooth muscle cell situated next to a blood vessel (*bv*). This cell contains a bilobed nucleus, one visible mitochondrion (*m*) and Golgi apparatus (*ga*, arrowhead). The area enclosed within the box outlined in black is enlarged in the inset, at left, to illustrate the profiles of contractile filaments cut in cross-section. Scale bar, 1 μm for A and B, 0.5 μm for the inset.

mately 30 nm in diameter and quite electron-dense. In some sections, the filaments appeared elongated, rather than circular, suggesting the filamentous nature of these structures. However, it was not possible to examine a single filament for a sufficient distance to estimate its total length. The filaments were generally surrounded by a second type of filament that was less electron-dense and substantially smaller, less than 10 nm in diameter. These filaments were similar in appearance, arrangement and dimensions to the thick (paramyosin- and myosin-

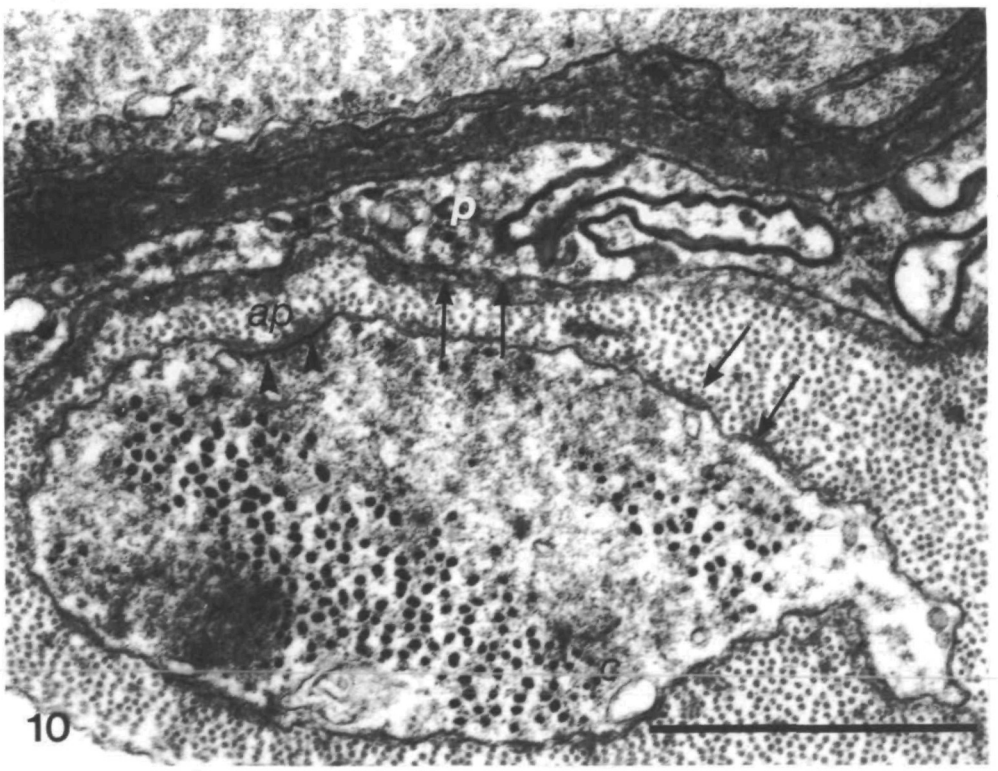

Fig. 10. Electron micrograph of a smooth muscle cell, showing attachment plate (*ap*, arrowheads) and cisternae (*c*) beneath the cell membrane. Both this muscle cell and the pericyte (*p*) of the adjacent blood vessel are surrounded by basal lamina (arrows). Scale bar, 1 μm.

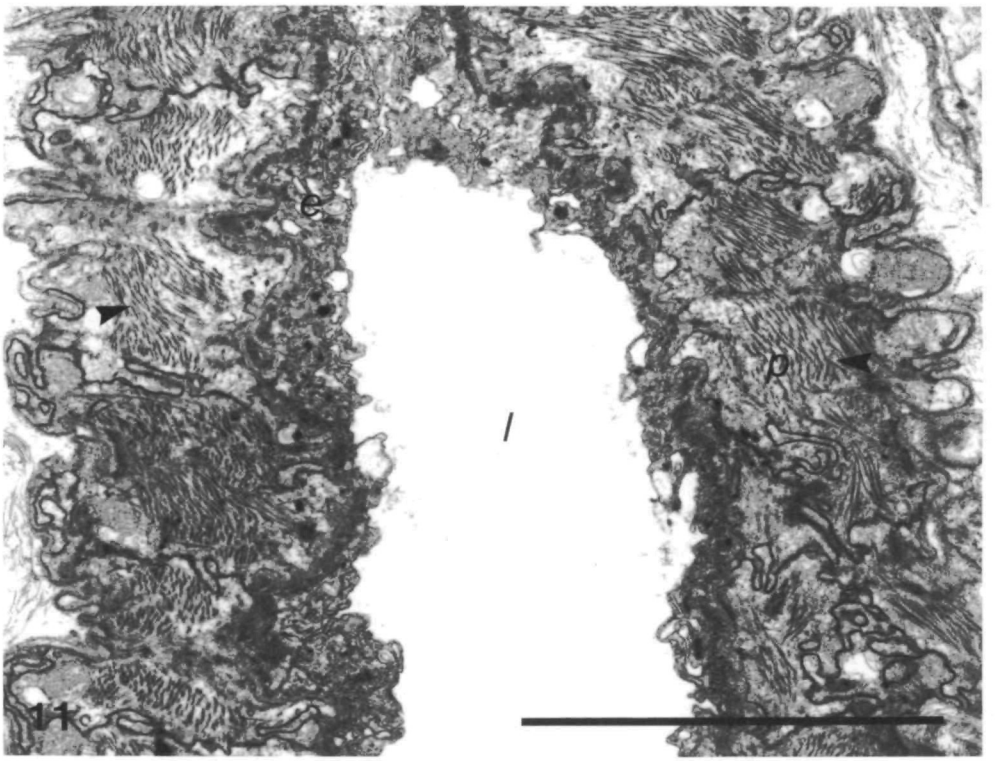

Fig. 11. Low-magnification electron micrograph of a large blood vessel cut in cross-section. The blood vessel wall, consisting of an inner layer of endothelial cells (*e*) and a more superficial layer of pericytes (*p*), surrounds the vessel lumen (*l*). The pericytes contain contractile filaments (arrowheads) and adjacent pericytes are connected by narrow, electron-dense pericyte junctions. Scale bar, 5 μm.

containing) and thin (actin-containing) filaments found in many molluscan contractile cells (Chantler, 1983).

In addition to the smooth muscle cells, the ganglion also had a second type of cell that contained arrays of contractile filaments. These cells were the pericytes that surround some of the larger blood vessels of the ganglion (Fig. 11). Although we did not perform a detailed study of the structure of these pericytes, their anatomy appeared to be similar to that of pericytes described in certain blood vessels of *Octopus* and *Sepia* (Barber and Graziadea, 1965, 1967; Browning, 1979). The filaments of the pericytes appeared similar in diameter to those of the ganglionic smooth muscle cells. These filaments were not visible in the pericytes of small-diameter blood vessels (Fig. 10). Adjacent pericytes were connected by electron-dense junctions (Figs 10 and 11).

Discussion

In this paper we have demonstrated that squid stellate ganglia contract. The

contractions of this ganglion are rather slow, with a mean frequency of $8\,min^{-1}$, and are usually very regular in their amplitude and frequency. These contractions are eliminated by several treatments that block influx through Ca^{2+} channels, but appear less sensitive to treatments that block Na^{+} channels. Thus, both Ca^{2+} and Na^{+} may be involved in generating contractions, but Ca^{2+} appears more critical. Electron microscopic studies of the stellate ganglion have identified two types of non-neural cells that contain an abundance of organized cytoskeletal elements and other morphological features characteristic of contractile cells. These cells may be the source of the contractions of the ganglion.

Because contractions could be recorded from isolated ganglia, it is clear that the contractile elements are intrinsic to the ganglion. In the ganglia of other molluscs, connective tissue sheaths have been reported to be the source of contractions (Rosenbluth, 1963; Mirolli and Gorman, 1968). Although the muscular epithelial layer covering the squid stellate ganglion is contractile (Miledi, 1967), it was not the source of the contractions we have described because this layer was removed from the ganglion. Squid giant axons have also been reported to undergo minute displacements during action potential generation (Tasaki and Iwasa, 1982). However, this is unlikely to be the source of the contractions that we have studied because: (1) although the ganglionic contractions are coordinated throughout the ganglion (Fig. 3), there is no obvious means of synchronously activating the neurons of the ganglion (Miledi, 1972); (2) tetrodotoxin blocks neural activity but does not affect the contractions; and (3) intracellular recordings from the giant axons reveal no electrical activity that is correlated with the contractions (G. Augustine, unpublished observation).

The most likely source of the contractions are the two types of cells that contain organized arrays of cytoskeletal filaments and are distributed throughout the stellate ganglion. One of these cell types, the pericytes of blood vessels, has been reported previously in the nervous tissue of other molluscs and has been proposed to endow blood vessels with contractile properties analogous to those of the arterioles of mammalian tissues (Barber and Graziadei, 1965, 1967; Sims, 1986). The second type of cell is a smooth muscle cell that has not been identified in other nervous tissues. Although junctions between pericytes have been described in other molluscan blood vessels (Barber and Graziadei, 1965, 1967; Browning, 1979), their function is unclear. If the junctions permit communication between pericytes, as has been proposed (Sims, 1986), they could provide the coordination needed to spread the signal for contraction throughout the stellate ganglion. It is also possible that the smooth muscle cells are coordinated by a mechanism that is not evident from electron microscopical observations. Thus, while the presence of appropriate structural specializations suggests that these cells are contractile, it is not yet known whether they do contract and whether such contractions could be coordinated to produce the synchronized movements we have described here.

Our ion substitution and pharmacological experiments indicate possible roles for Ca^{2+} and Na^{+} in generating the ganglionic contractions. One likely role for these ions is to generate the electrical activity that initiates the contractions; the

response of contraction frequency to the experimental manipulations should provide information on their site of action. Na^+ current appears to have some role in generating action potentials because contraction frequency was slowed in $0Na^+$(sucrose) saline. Ca^{2+} current apparently plays a more important role in production of spontaneous action potentials, because contraction frequency declined to zero in calcium-free saline and during treatment with inorganic Ca^{2+} channel blockers. This is consistent with studies indicating that action potentials with both Na^+ and Ca^{2+} components are usually found in slowly contracting muscles (i.e. cardiac and smooth muscle), including those of molluscs (Deaton and Greenberg, 1980).

The differential response of contraction amplitude and frequency to several experimental manipulations, such as Ca^{2+} channel blockers and Ca^{2+}-free saline, suggests additional actions beyond effects on electrical activity. In particular, the observation that DHPs sometimes decreased contraction amplitude without changing contraction rate indicates that these agents disrupt excitation–contraction coupling without significantly affecting the Ca^{2+} channels that generate electrical activity. This may reflect inhibition of a DHP receptor involved in excitation–contraction coupling (Rios and Brum, 1987). Although further work will be necessary to understand fully the Na^+ and Ca^{2+} requirements for ganglionic contractions, it appears that both ions are involved in generating electrical activity and that this activity initiates contraction *via* a DHP-sensitive coupling mechanism.

The function of the contractions of the stellate ganglion is unknown. One possibility is that the contractions play some role in the circulation of blood, as has been proposed for the contractions of the blood vessels of other cephalapods (Barber and Graziadei, 1965) and the cardiac ganglion of octopus (Alexandrowicz, 1963). Because invertebrates often have circulatory systems in which the heart is not the only circulatory pump, this could explain why endogenous contractile activity appears to be more obvious in invertebrate nervous tissue. Such peripheral pumping might be especially valuable for the large number of invertebrates that possess open circulatory systems. Alternatively, it is possible that the contractions are used for some other function that has not yet been identified. If so, perhaps closer scrutiny will also reveal the presence of endogenous contractions in vertebrate nervous tissue.

The most immediate benefit of our work has been to identify treatments that eliminate contractions of the stellate ganglion. These contractions have been a technical impediment to studies of transmission at the giant synapse of this ganglion and identification of several means of eliminating contractions could potentially help future studies. However, this goal is useful only if the treatments that eradicate contractions do not alter synaptic transmission. For example, while glutaraldehyde treatment blocks contractions of nervous tissues (Mirolli and Gorman, 1968; Kretz *et al.* 1986), this treatment is not useful at the squid synapse because it also eliminates synaptic transmission (G. J. Augustine, M. P. Charlton and S. J. Smith, unpublished results). Table 1 lists the treatments that we have

Table 1. *Effect of selected treatments on stellate ganglion contractions and transmission at the squid giant synapse*

Treatment	Effect on contractions	Effect on synapse
$0Na^+$(Tris)	Eliminated	Eliminated
$0Ca^{2+}$(Mg^{2+})	Eliminated	Eliminated
Ca^{2+} channel blockers		
Inorganic	Eliminated	Eliminated
Organic	Eliminated	No effect

See Materials and methods for details of saline solutions.

found to block ganglion contractions, as well as the known or anticipated effects of these treatments on synaptic transmission. Na^+ removal should eliminate transmission at the giant synapse because the postsynaptic response is Na^+-dependent (Llinas *et al.* 1974). Ca^{2+}-free saline (Miledi and Slater, 1966) and inorganic Ca^{2+} channel blockers (Augustine *et al.* 1989) block transmission by eliminating presynaptic Ca^{2+} influx. However, DHPs do not alter synaptic transmission (Augustine *et al.* 1989) even though they eliminate contractions (Fig. 8). Thus, treatment with DHPs offers a means of selectively eliminating contractions. One study has already taken advantage of our findings by using DHPs to permit movement-free optical measurements of Ca^{2+} accumulation in the giant synapse (S. J. Smith, J. Buchanan, L. Osses, M. P. Charlton and G. J. Augustine, in preparation). It is likely that DHPs will be of value in other applications in the future.

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