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Gap Junctions: Their Presence and Necessity in Myometrium During Parturition

Abstract. *Thin sections of longitudinal and circular muscle of myometrium obtained from rats during pregnancy, at term, during delivery, and postpartum were quantitatively examined in the electron microscope. Gap junctions (low resistance pathways) were only present during or immediately prior to delivery and immediately postpartum. The absence of gap junctions during gestation may be necessary for maintenance of pregnancy, while their occurrence during parturition may lead to effective termination of pregnancy.*

Nexuses or gap junctions appear as a fusion of membranes into five or seven layers in ultrathin sections of tissue, or as aggregates of membrane particles when examined by freeze-fracture electron microscopy. They are thought to provide sites of low resistance to current flow between electrically excitable cells (*1*), including smooth muscle (*2*), and thus to be the pathway for conduction of action potentials or applied pulses (*1*). However, these junctions have not been demonstrated in several types of smooth muscle known to be electrically coupled (*3, 4*). In previous studies of the ultrastructure of rat myometria, we have not found even a single nexus or gap junction between smooth muscle cells in tissues from pregnant animals or those treated with estrogen or progesterone, or both (*3*). However, nexuses occurred frequently between the fibrocytes which surround the muscle bundles (*3*).

Recently, we found gap junctions between smooth muscle cells of the longitudinal and circular muscle layers of rat myometria that had been fixed for electron microscopy (*5*) during parturition (see Fig. 1). We have now quantitatively examined (*6*) the presence of gap junctions in thin sections of rat myometrium obtained during pregnancy, at term, during labor, and postpartum (Table 1).

There were no nexuses or gap junctions between muscle cells in either the longitudinal or circular muscle layers in tissues from animals fixed on approximately day 14 of gestation, or in any tissue from immature animals including animals injected with estrogen or progesterone, or both (Table 1). In all the tissues studied, there were many intermediate and close contacts between muscle cells, and frequent gap junctions between fibrocytes, as seen previously (*3, 4*).

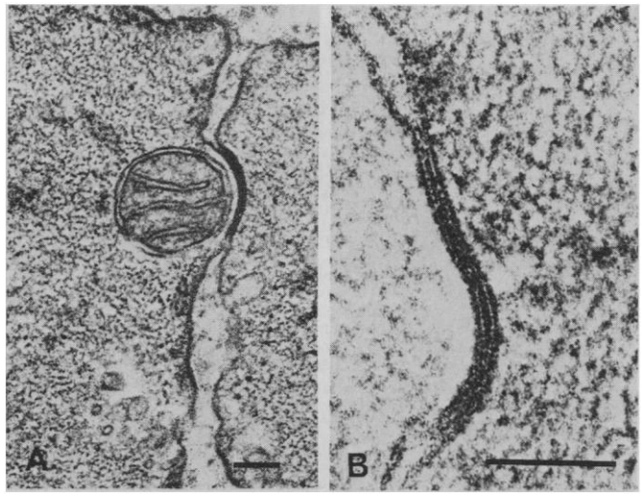

Fig. 1. (A) Electron micrograph of gap junction between two smooth muscle cells of the longitudinal muscle layer of rat myometrium fixed by intra-arterial perfusion during parturition (scale bar, 0.1 μm). (B) Gap junction between muscle cells from tissue similar to that in (A), as shown at higher magnification (scale bar, 0.1 μm).

In some tissues fixed at term (21 to 22 days) gap junctions were not observed in either the longitudinal or circular muscle layers (Table 1). However, in tissues from other animals gap junctions were present in both muscle layers. Gap junctions were always present in both muscle layers when they had been fixed during parturition (when 1 to 12 fetuses were delivered). Myometrial tissues from animals fixed 1 to 8 hours postpartum also always contained gap junctions. The numbers of gap junctions in animals delivering compared to animals not delivering or postpartum was insignificant ($P > .05$). The mean diameter of gap junctions in the muscle layers increased significantly in animals delivering ($P < .01$) or postpartum ($P < .05$) compared to animals at term. The corresponding areas of gap junctions relative to total membrane areas also increased (data not shown). There were no significant differences ($P > .05$) between mean lengths during delivery compared to postpartum.

That gap junctions are visible in thin sections between smooth muscle cells exclusively at term and during or following parturition has important implications for the maintenance and termination of pregnancy. Throughout the gestation period, the absence of gap junctions between contractile cells may be a major factor in the maintenance of pregnancy by preventing electrical communication between cells and coordinated contractions. The appearance of gap junctions between smooth muscle cells immediately prior to parturition may provide a large membrane area of low resistance allowing spread of electrical information between cells and terminating pregnancy by synchronizing uterine contractility for effective expulsion of the fetus.

We believe that gap junctions are present in tissues at term only when the animals have entered the final stages of gestation, that is, are ready to deliver. This hypothesis is supported by the finding that some animals reputedly at term cannot be induced to deliver by oxytocin while some can; only the latter have gap junctions in myometria. Since the time of onset of readiness to deliver (labor) of a particular animals varies, the occurrence of junctions will also vary in animals supposedly at term. This would explain our failure to find gap junctions in all animals reputedly "at term" (Table 1).

Measurements of space constants by microelectrodes during pregnancy and parturition should indicate the extent of electrical coupling. However, these parameters for the various muscle layers of the rat uterus have not been adequately defined before term, at term, and during parturition. In the rabbit uterus, Csapo (*7*) has shown in situ with implanted electrodes that propagation of electrical activity is greatest during parturition.

Rat uterine smooth muscle strips from both pregnant and nonpregnant animals behave as an electrical syncytium or a leaky cable over short distances as measured in vitro (*8*), and the cable theory has been applied to the tissue (*9*). Low-resistance contacts between smooth muscle cells are assumed to be necessary if the cable hypothesis is applied, but gap junctions were not observed in these tissues, suggesting that electrical coupling in such cases may be dependent on the presence of intermediate or close apposition contacts (*3*, *4*) between cells. Since cable properties of uterine muscle have been studied in vitro, it is possible that gap junctions form or aggregate in vitro perhaps because of the loss of some humoral agent which prevents their formation in vivo. However, we have never found gap junctions in pregnant myometria removed prior to term and incubated in vitro (*10*). There is also the possibility that small gap junctions seen with freeze-fracture methods (*11*) may be present between cells and these may not be visible within our thin sections. If so, the final stage of gestation is accompanied by the assembly of small gap junctions into large ones. Studies with freeze-fracture methods are required to determine whether the creation of new or assembly of small gap junctions occurs at term. Our studies with these methods indicate the presence of typical gap junctions in tissues from parturient animals, in agreement with our data from thin sections which show conclusively the rapid formation of large gap junctions either just prior to or during parturition. We suggest that this formation is essential prior to normal labor and if it occurs prematurely it may lead to irreversible premature labor.

Among the events prior to parturition that could be responsible for stimulating the structural changes in the myometrial smooth muscle cell membrane leading to gap junction formation are changes in the concentrations of progesterone, estrogen, oxytocin, or prostaglandin. One event that might initiate formation of large gap junctions is progesterone withdrawal. Indeed, the inhibition of the formation of gap junctions until parturition may be the basis for the progesterone block hypothesis advanced by Csapo (*7*). There is convincing evidence that changes in plasma progesterone levels in some animals are related to the maintenance and termination of pregnancy (*7*). We propose that gap junction formation is a necessary precondition for the coordinated contractions of labor and is controlled by a signal such as progesterone withdrawal.

Evidence to support progesterone withdrawal as the controlling factor in gap function formation comes from the following observations: (i) ovariectomy of pregnant animals at midterm results in abortion or resorption of fetuses and the rapid appearance of gap junctions; both abortion and gap junction appearance are prevented by progesterone administration; and (ii) gap junctions appear in both gravid and nongravid horns during delivery in animals unilaterally ovariectomized prior to conception. Progesterone administration to animals at term may prolong or prevent the appearance of gap junctions and delay or prevent delivery. The failure of progesterone withdrawal to induce gap junctions in nonpregnant animals (Table 1) indicates that other factors may also be involved.

It is not known whether gap junctions develop in the myometrium of other animals, including humans, during parturition. If they do, studies of the conditions that control their formation may lead to a better understanding of the physiology of parturition as well as a more rational basis for developing and evaluating drug therapy used to prevent and terminate pregnancy or delay premature labor.

Table 1. Number and length of gap junctions (GJ's) in myometrium obtained from rats during pregnancy and postpartum, and from immature rats. Tissue samples were obtained from longitudinal and circular muscle of myometrium. The number of pieces of tissue examined (one section per uterine horn, and approximately equal numbers from longitudinal and circular muscle) is shown by *N*. The number of GJ's indicates the total of five- or seven-membered junctions found within 8 to 12 electron micrographs of each piece of tissue. Distance shows the length of the membrane measured from electron micrographs of smooth muscle cells in transverse orientation. Hormones were injected singly or together as follows: estrogen (estradiol) (50 μg) and progesterone (2 mg) daily for 15 days; estrogen (50 μg) only was injected for 1 to 3 days in one group after the 15-day treatment with both hormones.

Time at which tissue was obtained	*N*	GJ's (No.)	Distance (μm)	Junctions per 1000 μm	Mean* length of GJ's (μm)
During pregnancy					
Day 14	7	0	4770		
Day 21 to 22 (at term)					
With GJ	23	62	14,019	4.42	0.15 ± 0.11
No GJ	11	0	6897		
Day 22 to 23	14	50	7955	6.29	0.20 ± 0.10†
Postpartum					
1 to 8 hours	6	14	3334	4.20	0.25 ± 0.13‡
Day 16	6	0	4739		
Immature					
Control	3	0	2302		
After hormone injections					
Estrogen	3	0	1979		
Progesterone	3	0	1986		
Estrogen plus progesterone	3	0	2202		
Estrogen plus progesterone plus 1 to 3 days of estrogen only	3	0	2130		

*Mean ± standard deviation. †Significant differences ($P < .01$) between mean lengths of gap junctions in tissues from animals at term and at delivery (unpaired *t*-test). ‡Indicates significant differences ($P < .05$) between tissues at term and postpartum.

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Fixation of tissues in situ for electron microscopy was accomplished by (i) intra-arterial perfusion with glutaraldehyde as used previously (*3*) or (ii) filling the peritoneal cavity with the fixative. After 20 to 30 minutes of fixation, the animals were killed and tissue was removed from the middle of each horn. These tissues were fixed (2 percent glutaraldehyde and 4.5 percent sucrose in 0.075*M* cacodylate buffer, *p*H 7.4) for an additional 2 hours. After the initial fixation in glutaraldehyde, the tissues were washed in buffer for 60 minutes and fixed for another 90 minutes in 1 percent osmium tetroxide (in cacodylate buffer, 0.05*M*, *p*H 7.4). All tissues were stained en block with saturated uranyl acetate for 30 minutes, dehydrated in graded alcohols, and embedded in Spurr. The tissues were oriented in molds so that either the outer longitudinal or inner circular muscle layers would be cut in cross section.

6. Eight to 12 nonoverlapping photographs from one section of each tissue were taken of smooth muscle cut in cross section and examined at ×11,000. The negatives were enlarged three times (×33,000) and printed on 20 by 28 cm paper. A map tracing compass (Selsi, West Germany) was used to trace along the surface of each smooth muscle cell in the photograph. Each suspected gap junction observed in the photograph at ×33,000 was further enlarged to ×100,000 magnification for identification and length measurements. Cell-to-cell contacts were identified as nexuses or gap junctions at the higher magnification if they showed a five-lined structure or a seven-lined structure with a 2-nm gap between outer membrane leaflets.
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Optical Transforms and the "Pincushion Grid" Illusion?

Schachar (*1*) has presented experimental evidence which he contends contradicts the theory that the visual system performs two-dimensional Fourier transformations of observed patterns. He does this on the basis of the "pincushion grid" illusion in which diagonal lines are seen when one views a square grid of lines. Schachar presents what he claims to be an optical transform of the grid, and since no diagonal components appear in his optical transform, he contends that the visual system does not perform a Fourier transformation of the observed grid.

In this communication I raise two objections to Schachar's report.

First, his optical transform is not that from a grid. The optical transform, or optical diffraction pattern, of a square grid is well known to be a square array of spots. The theory is developed in many standard texts [see, for example, Ditchburn (*2*)] and is easily demonstrated with a laser. Figure 1 is a photographic enlargement of a grid whose actual spacing is 0.085 mm. The beam of a helium-neon laser was directed onto the grid, and the diffraction pattern, intercepted by photographic paper at approximately 2 m from the grid, is reproduced in Fig. 2. The spot pattern is clearly evident and includes diagonal components.

The photograph presented by Schachar in his figure 1 does not exhibit any of the spots characteristic of the diffraction pattern of a grid, neither on the major axes nor on the diagonal. It appears to be the pattern observed when a laser beam passes through photographic film of approximately even density. Schachar does not give the spacing of the grid used for the diffraction experiment, but if the cover of *Science* is a one-step photographic enlargement of the 35-mm slide mentioned in the article, then the actual grid spacing on the slide would have been on the order of 1 mm, about the size of a typical laser beam, and there would not have been a repeating object to produce a diffraction pattern.

Second, a more fundamental question arises concerning the applicability of optical diffraction patterns as an analogy for the Fourier transformation that is postulated to occur in the visual processing system. A diffraction pattern is not a Fourier transform, but is the complex square of the Fourier transform and is not unambiguously related to the diffracting object. It is this ambiguity that makes the determination of crystal structure from x-ray diffraction patterns a complicated task.

One specific form of this ambiguity relates to an observation by Schachar. He mentions that "a negative of the pincushion grid (black pincushions separated by white spaces) produces the illusion of diagonal black lines." If the visual system uses diffraction transforms, not true Fourier transforms, this observation of the reversal of the color of the illusion would be in contradiction to Babinet's principle (*3*), which states that complementary objects, that is, those that have reversed contrast, give identical diffraction patterns. Schachar's observation of reversed color of the illusion when complementary grids were used, violates Babinet's principle and demonstrates that the visual system does not behave as though it used diffraction transforms. This neither proves nor disproves that Fourier transforms are used by the visual system, but it does show that Schachar's basic assumption, that an optical transform can be used as an analogy for possible Fourier transformations in the visual system, is not valid.

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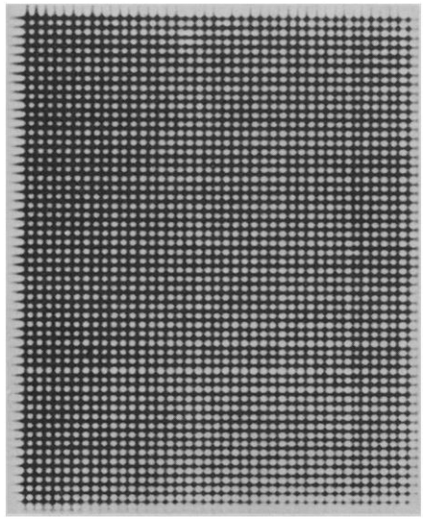

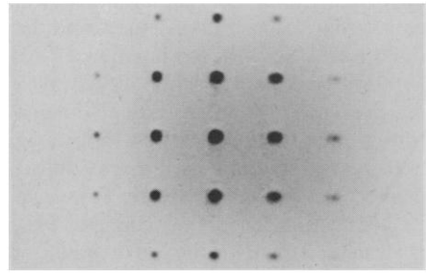

Fig. 1 (left). Photographic enlargement of a diffraction grating. The grating was produced by ruling black lines on white paper and producing a photographic reduction on a glass-supported emulsion. The actual spacing was 0.085 mm. Fig. 2 (right). An optical diffractión pattern produced by directing a helium-neon laser beam through the grating produced in Fig. 1.

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Schachar (*1*) failed to observe diagonal spatial frequencies in the optical Fourier transform of a "pincushion grid." This implied that the illusion of diagonal lines that are observed in such a pattern cannot be attributed to two-dimensional Fourier analysis by the human visual