Intercellular communication in smooth muscle

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Abstract. The functioning of a group of cells as a tissue depends on intercellular communication; an example is the spread of action potentials through intestinal tissue resulting in synchronized contraction. Recent evidence for cell heterogeneity within smooth muscle tissues has renewed research into cell coupling. *Electrical coupling* is essential for propagation of action potentials in gastrointestinal smooth muscle. *Metabolic coupling* may be involved in generation of pacemaker activity. This review deals with the role of cell coupling in tissue function and some of the issues discussed are the relationship between electrical synchronization and gap junctions, metabolic coupling, and the role of interstitial cells of Cajal in coupling.

Key words. Gap junctions; intercellular junctions; field coupling; canine colon; dye coupling; electrical coupling; metabolic coupling.

Introduction

Within smooth muscle layers, perfect synchronization of cellular activity occurs during generation and propagation of phasic contractile activity. Because of the marked heterogeneity in cell types[24, 32] and in the structural aspects of cell coupling within a muscle layer, it is intriguing to resolve the mechanisms of cellular communication. Cellular communication allows groups of cells to function as a tissue; it is therefore important to comprehend and appreciate the physiology and pathophysiology of cell coupling. This review will discuss different types of cell coupling and methods to study them. It will also discuss a novel aspect of cell coupling: the role of interstitial cells of Cajal. Furthermore, this review will point out important questions to be resolved. Before a discussion on intercellular communication, in particular electrical and metabolic coupling, can develop, an overview of the structural aspects of cell to cell coupling is essential.

Structures responsible for intercellular coupling

A variety of structural arrangements exist that facilitate communication between smooth muscle cells. The best-studied structure is the gap junction. Other structures include close apposition membranes, ball-and-socket type appositions, and intermediate-type contacts.

Gap junctions

Gap junctions are specialized regions of cell contact where a hexameric configuration of identical protein subunits (connexins) surround a central pore which is aligned with an identical structure in the adjacent cell membrane. The hexagonally arranged connexins form a connexon, 6–7 nm in diameter. Paired connexons form a continuous channel with a diameter of 1.5–2 nm. Channels often cluster together; in the guinea pig ileum ~ 250 gap junctions would occupy ~ 0.02 μm^2 or ~ 0.21% of the cell surface[50].

A variety of techniques exist to recognize gap junctions. The most widely used technique is the examination of thin sections of fixed tissue with the electron microscope. Gap junctions are characterized by a typical seven-line structure[14, 22]. The strength of this method is that gap junctions between cells can be positively identified. Furthermore, the connecting cells can be identified, and some specific characterization can be done, such as estimation of the length of the gap junction and estimation of frequency of gap junction contact. The limits of the technique are that it cannot recognize very small gap junction contacts, and only in a theoretical sense could it prove absence of gap junctions since it would depend on extensive serial sectioning and it would require that neighbouring cells never approach each other very closely.

Gap junctions can also be identified through the electron microscope using freeze fracture replicates; this reveals the presence of connexons as assemblies of membrane particles protruding usually from the protoplasmic surface of the membrane[14]. The freeze fracture technique usually does not identify gap junctions on cell protrusions since fracture planes do not follow the contours of these protrusions. Identification of small gap junctions or connexins is difficult since membrane particles do not identify themselves as connexins. Also, identification of gap junction particles does not prove a gap junction structure connecting two cells[14].

Gap junctions can be made visible by immunohistochemical staining using antibodies against gap junction proteins. When we applied this technique (with antibody to connexin 43) to the canine colon musculature, examined extensively by electron microscopy, we confirmed the EM findings. For example, the submucosal surface of the circular muscle layer was extensively stained whereas the longitudinal muscle did not show any staining. Similarly, rat jejunal circular muscle was intensely stained compared to absence of staining in the longitudinal muscle[20]. Immunohistochemical staining of gap junction protein using antibody beautifully demonstrates gap junctions at a light microscopic level. Its resolution at

both the light and the EM level is not fully appreciated yet. It may be difficult to identify single protein membrane connections.

Gap junction protein can be identified by western blot techniques. It has been observed that in tissue in which gap junctions were not identified by EM, gap junction protein was found [5]. This is an important observation for which the implications have not been fully investigated – it may be that the gap junction proteins are not assembled in patches of a size identifiable by EM, or they may all be in a non functional state, possibly ready to be made functional upon an appropriate stimulus.

Probes for identification of mRNA or DNA coding for gap junction proteins have become available and they indicate heterogeneity amongst gap junction connexins [33]; connexin 43 is prominent in cardiac, vascular [33, 39] and intestinal smooth muscle [20]. While northern blot analysis is a sensitive assay for the presence of mRNA, it does not reveal presence of functional gap junctions. Indeed, several nonvascular cell types, expressing abundant amounts of connexin 43 mRNA, did not show any lucifer yellow transfer [32].

One of the most fascinating aspects of gap junctions is how their presence and conductive state is controlled. The potential for modulation of cell to cell communication exists at several stages, including the synthesis of connexin, its insertion into the plasma membrane, the formation of assembled plaques, the regulation of channel function and the removal and degradation of the junctions.

In myometrium, gap junctions can barely be found using EM techniques up to the last day of pregnancy; while one day later during delivery, mRNA for connexin 43 has gone up [42] and gap junctions are present in high density [14, 19]. Gap junctions can also be induced by pharmacological means: the potassium channel blockers 4-aminopyridine and tetraethyl ammonium caused increase in gap junctions assessed by electron microscopy in the canine trachealis muscle [30].

Regulation of gap junction conductance has been extensively studied. Transfer of deoxy glucose through gap junctions in myometrial smooth muscle was markedly reduced upon increase in intracellular cAMP [11]. In contrast, cardiac myocytes, also expressing connexin 43, exhibited an increase in junctional conductance in response to cAMP [51]. Junctional conductance in cardiac myocytes decreases in response to cGMP; regulation may depend on phosphorylation of the cytoplasmic domains of the connexin proteins [51]. In addition to cyclic nucleotides, both intracellular calcium levels [22] and the intracellular pH can markedly affect gap junction permeability [22, 54]. The physiological importance of such regulation remains to be elucidated. This may not be easy to achieve, particularly in the light of the fact that physiological calcium or pH transients are often very shortlasting and may be located in areas of the cell away from the gap junctions. Gap junction conductance may also be regulated by voltage. The establishment of a 30 mV potential difference between two amphibian embryonic cells resulted in 95% reduction in gap junction conductance and in loss of dye transfer [52]. However, in other cell types, such as cardiac myocytes [51] or pancreatic β-cells [40] voltage-dependent gap junctional conductance changes were small or absent.

Gap junctions can play an important role in protection of tissue against local injuries, in that closure of gap junctions can prevent spread of injury. In myocardial infarction, the coupling between a healthy and a damaged muscle cell is markedly decreased, possibly through a calcium-dependent mechanism [22].

Consistent with the idea, to be developed later in this paper, that a primary role for gap junctions may be non-electrical in nature, is the observation that gap junctional communication is found in almost all non excitable cells [22]. Suggested roles for gap junction communication in such systems include control of growth and differentiation, and synchronization of hormonal control.

A primary function of gap junctions, or the presence of cytoplasmic communication, may be that it increases the reliability of tissue function [21]. The absence of cytoplasmic communication might lead to a great variability of resting behaviour of cells or variability in response to stimuli. A stable membrane potential, if dependent on a relatively low number of K channels, could only be achieved by coupled cells [21, 41]. When single cells were to be isolated within a tissue, shortage of a crucial intracellular messenger might render the cell ineffective. Aggregation of such cells by permeable junctions with healthy cells could alleviate shortcomings.

Cell to cell contacts other than gap junctions

Smooth muscle cells have at least 3 types of contact other than gap junctions. These are usually classified as 1) close or simple membrane appositions, 2) intermediate contacts, and 3) 'ball and socket' contacts, sometimes called interdigitations [18].

Areas of very close approximation of cell membranes (10 nm or less) are called *close appositions*. These contacts do not seem to have specialized features and have not been attributed to any structure seen in freeze fracture replicates of the membrane. Close appositions can occur as point contacts (fig. 1). Such contacts may be analogues to very small gap junctions but because of their small size they cannot fulfil the electron microscopy criteria of a gap junction (i.e. the 7-line structure) [18]. Close appositions can also occur over remarkably constant large areas of apposition, usually with no specialized structure (such as increased density), neither at the cytoplasmic surfaces, nor in the narrow interval between the apposed membranes. Contacts between the interstitial cells of Cajal in human small intestine are one example [43]. *Intermediate contacts* are junctions characterized by an increased density of the cytoplasm in the apposing

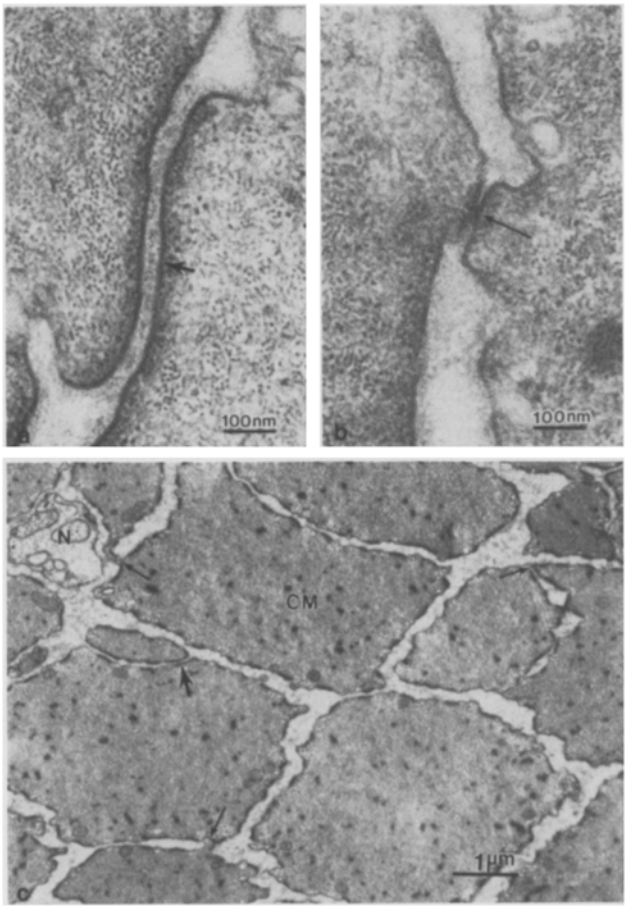


Figure 1. Electron micrographs of cell-to-cell contacts between smooth muscle cells from the circular muscle layer of canine colon, as seen in the thin sections. *a* A high magnification micrograph of an intermediate contact (thick arrow) (≈ 35–40 nm). Note the increased density of the cytoplasm in the apposing cell membranes, as well as in the extracellular space. × 65,000. *b* A high magnification micrograph of a close apposition contact (less than 10 nm) (thin arrow) between two smooth muscle cells × 65,000. *c* A low magnification micrograph of a cross section through the circular muscle layer showing close appositions contacts (thin arrows) and an intermediate contact (thick arrow). N, small nerve bundle. × 8500.

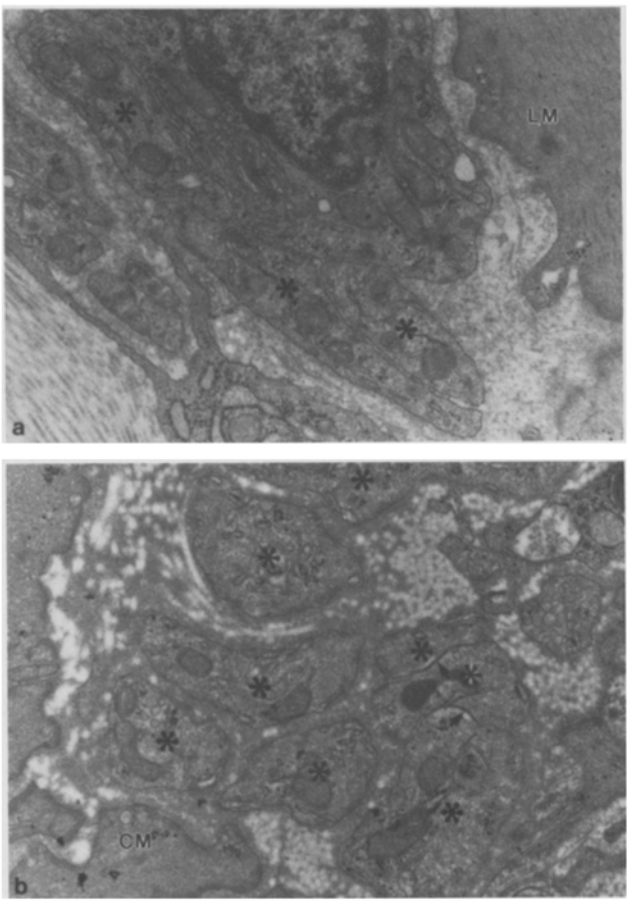


Figure 2. Cell-to-cell contacts in two networks of interstitial cells of Cajal (ICC) associated with the musculature of the rabbit small intestine. Upper micrograph: ICC processes (*) in the ICC network within the Auerbach's Plexus of the duodenum. Cell-to-cell contact through simple membrane appositions. At lower left, one can see a fibroblast enveloping 5 small ICC processes. At right, a longitudinal muscle cell (LM) is situated. × 18,300. Lower micrograph: ICC processes (*) in the ICC network within the deep muscular plexus of the jejunum. Cell-to-cell contact through gap junctions (arrows). At left circular muscle cells (CM) are situated. × 18,300.

cell membranes as seen in thin sections (fig. 1). The intercellular space is 20–100 nm wide. It has been suggested that the structure of the intermediate contacts is similar to the intercalated disc found between cardiac cells; they may serve as a mechanical support[18]. Intermediate contacts can occupy a major space between the longitudinal domains of smooth muscle cell membranes. Caveolae may occur at both sides opposite each other. There is often accumulation of dense, fine filamentous material between the membranes, with extracellular microfibrils embedded in intercellular material. Comparison of relaxed with contracted smooth muscle suggests a high degree of flexibility of the intermediate contacts. *Ball and socket contacts* are often observed where a short and blunt process from one cell protrudes into a pocket shaped invagination of the neighbouring cell[17]. Close apposition contacts can be found within a ball and socket contact.

The functions of the various contacts have not been resolved. Structures with apparently similar functions may have markedly different cell to cell contacts. For example, in rabbit small intestine one subset of interstitial cells of Cajal (ICC-AP, associated with Auerbach's plexus, putative pacemaker cells) do not show recognizable gap junctions, but form bundles of closely associated, overlapping cells and cell processes (fig. 4), while another subset (ICC-DMP, associated with the deep muscular plexus of the circular muscle layer) lacks this specific organization, but is coupled by numerous, large gap junctions between constituent cells (fig. 2).

Metabolic coupling

Metabolic coupling, or metabolic cooperation, can be defined, according to Hooper and Subak-Sharpe[23] as: "the exchange of molecules between cells through permeable junctions formed at sites of cell contact". Metabolic

coupling may be critical for nutrient supply for cell survival in tissue that is poorly vascularized [23]: the cortical and nuclear fibre cells in the vertebrate lens do not have direct access to nutrients from the vasculature; intercellular transport through extensive gap junction contact regions seems to be the pathway for nutrient supply [22]. Metabolic coupling may play a role in synchronization of hormonal stimulation where hormonally triggered changes in intracellular second messengers may be transmitted to other cells through gap junctions. This was beautifully demonstrated by co-culturing of myocardial cells and ovarian granulosa cells; these cells formed gap junction contacts with each other in culture [34]. Myocardial cells, normally unresponsive to follicle stimulating hormone (FSH), responded in co-culture to FSH in a way that mimicked the normal response to norepinephrine. Since actions of both norepinephrine and FSH are mediated by intracellular cAMP, gap junction transfer of cAMP was most likely underlying the phenomenon.

Metabolic coupling requires the passage of small molecules from one cell to another without passing the extracellular space. Hence, metabolic coupling can be measured by the study of transfer of small molecules that are either radioactive or fluorescent. The former has been successfully employed by Garfield and co-workers in uterine smooth muscle. Cole and Garfield [11, 12] provided evidence for an increase in metabolic coupling after gap junction formation in the rat myometrium during parturition when they saw a marked increase in the spread of radioactive 2-deoxy glucose. This intercellular communication pathway was modulated by intracellular cAMP. In the same study, longitudinal muscle of the portal vein did not show gap junctions nor spread of 2-deoxy glucose. Fluorescent dye coupling was successfully employed by Blennerhassett and co-workers [8, 9]. Rat aortic smooth muscle cells in culture [9] as well as intact rat uterine longitudinal muscle [8] (fig. 3) were injected with lucifer yellow which was seen to diffuse into neighbouring cells. The intensity of the fluorescence was progressively weaker with increasing distance from the injected cell. Thus, the development of a three-dimensional staining gradient, seen in 3-D but necessarily recorded in 2-D by photographic means, indicates the three-dimensional nature of a gap junction coupled network. This is dramatically demonstrated in the work of Caveney and co-workers [44] in studies of cell coupling in an epithelial sheet, where the development of 'steps' in intensity at cell boundaries are quantified. In these and other studies, there is a specific criterion for a gap junctionally coupled tissue, namely that a gradient of fluorescence intensity develops. For a given tissue, the level of coupling can be related to the time it takes to develop the gradient. In comparison between systems, different numbers of gap junctions may also be present. The absence of a dye spread may then indicate that there is no metabolic coupling between cells, or that it is below the threshold of detection. Full staining of two adjacent cells without any further spread may indicate an 'artifact', in that the microelectrode through which infusion takes place may have penetrated a second cell due to vibration, inadvertent movement or tissue contraction. Since dye injection takes a considerable amount of time, this does happen in practice. In such cases where coupling is low or thought absent, it is important that the membrane potential remain unchanged since a change would indicate an artifactual situation. As an example, one can compare dye injection into uncoupled tissue in figure 12 of reference 9 (Blennerhassett et al.) with that in a poorly coupled tissue in figure 5 of reference 8 (Blennerhassett and Garfield).

Figure 3. Lucifer yellow spread in rat myometrium: the criterion for good metabolic coupling is that a gradient of fluorescence intensity develops. Note the step-like decrease in intensity of fluorescence in successive cells away from the source cell in this well-coupled tissue: myometrium of a delivering rat where the number of gap junctions is high. This is a qualitative demonstration of how gap junctions permit the diffusion of molecules between cells, away from a locally high concentration. × 360.

Although the major role of gap junctions is thought to be the coupling of cells into an electrical syncytium, its role in metabolic coupling may be underestimated. The dramatic increase in gap junctions in myometrium during labour may be related to synchronization of hormonally induced activity rather than improved electrical coupling (see below). Recently, evidence was obtained that pacemaker activity in intestinal smooth muscle is regulated by oscillations in intracellular metabolites [28]. Metabolic coupling may therefore play a role in generation of gastrointestinal pacemaker activity. In most gastrointestinal tissues, the location of the origin of generation of action potentials coincided with the presence of a network of interstitial cells of Cajal [2, 6, 35, 36, 56]. A characteristic of this network is the abundant intercellular coupling by gap junctions consistent with metabolic coupling.

Although outside the scope of this review, communication through the extracellular medium involving products of intracellular metabolism is well established. In mammalian tissue, neuronal communication depends on it and cytokines can function as a signal between different immunological cell types [21]. Synchronized oscilla-

tions in movement of *Dictyostelium discoideum* depends on secreted cAMP and extracellular cAMP receptors[27]. β cells within an islet form clusters of gap junctionally coupled cells[37]. Synchronization of cells within an islet was postulated to be due to cyclic ion concentration changes in the extracellular space[37]. Similar mechanisms may be important in coordination of cellular activities in smooth muscle.

The existence of metabolic coupling proven with dye coupling will also indicate the existence of electrical coupling for obvious reasons. However, the absence of dye coupling may not mean the absence of electrical coupling. Meda et al.[37] observed that only a fraction of the electrically coupled pancreatic β cells showed dye transfer with lucifer yellow.

Electrical coupling

Phasic contractions in intestinal smooth muscle are a consequence of the generation of action potentials, synchronized within circumferentially oriented muscle sheets (lamellae); this requires intercellular communication between the pacemaker loci (including interstitial cells of Cajal)[24] and the smooth muscle cells, and between muscle cells in the lamella. Electrical coupling between cells occurs when the electrical activity of one cell influences that of the other through passive propagation of an electrical event, active propagation of action potentials, or induction of synchronization of electrical oscillations such as pacemaker activity, slow waves or spike-like action potentials. For passive electrotonic current spread, based on local circuit theory, physical connections between membranes of adjacent cells, which are provided by gap junctions, are required. Active and passive propagation of action potentials, or synchronization of electrical activities may occur via the electrical pathway created by gap junctions; in addition electrical activity can also be induced or synchronized across close apposition membranes through the development of an electrical field in a narrow cleft between the membranes of adjacent cells – a process called ephaptic or field coupling[3,45,49,53].

Electrical coupling can be measured in different ways. One method could suggest poor coupling (e.g. poor electrotonic current spread) while another method on the same tissue could suggest good coupling (synchronization of electrical activity). It is therefore important to emphasise the parameter measured and method of measurement when discussing electrical coupling.

Synchronization of electrical activities as measured at different spots in a tissue

Two electrodes can simultaneously record action potentials generated at different sites. If the action potentials are synchronized with a constant phase relationship one can conclude excellent electrical coupling. When the activities are not synchronized, one can speak of poor electrical coupling[4]. Although this method indicates whether or not cells at the two recording sites are electrically coupled, it does not reveal the mechanism of intercellular communication. The myometrium before parturition showed effective electrical coupling using extracellular electrodes covering relatively large distances (fig. 4)[38]. Action potentials were seen to be synchronized over a distance of 30 mm exhibiting a constant phase lag and a propagation velocity of 8 cm/s. Gap junctions are reported to be absent or rare in myometrium before parturition[38]. After the appearance of abundant gap junctions during labour, the propagation velocity increased to 14 cm/s. However, there is little if any qualitative differ-

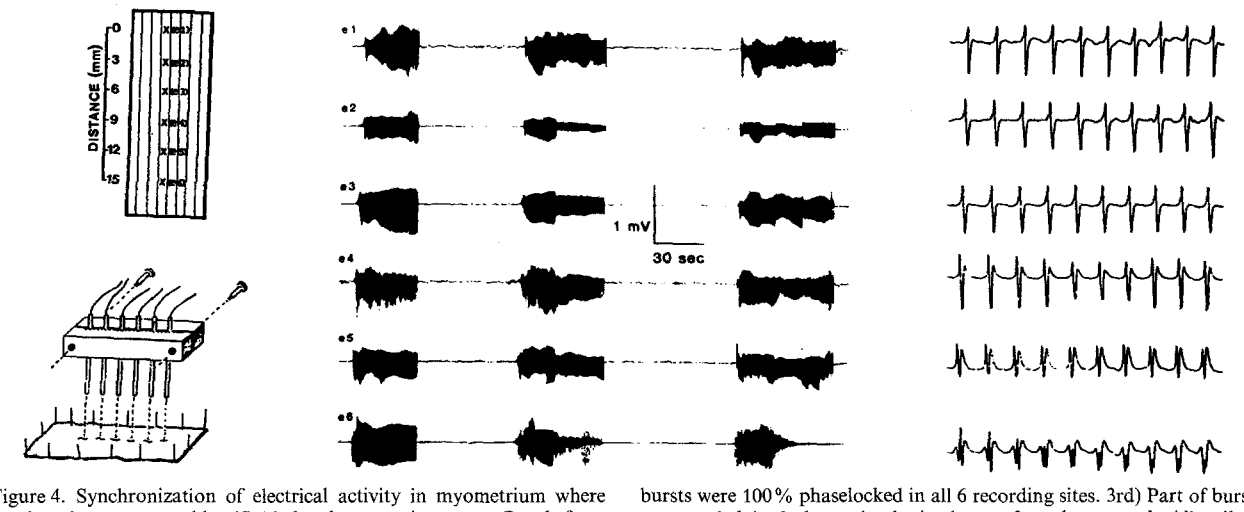


Figure 4. Synchronization of electrical activity in myometrium where gap junctions were not identifiable by electron microscopy. Panels from left to right: 1st) Arrangement of electrodes and distance between them. Six electrodes were positioned with a 3 mm distance between each pair, parallel to the long axis of longitudinal muscle fibres. 2nd) Apparent axial propagation of burst discharges in longitudinal segments at preterm. The bursts were 100% phaselocked in all 6 recording sites. 3rd) Part of burst as recorded in 2nd panel, obtained at a fast chart speed. All spikes recorded propagated over the entire recording area, at continuous phase lag, with a propagation velocity of 7.9 ± 3.0 cm/s.
Composite figure from Miller et al.[38], reproduced with permission.

ence between synchronization of action potentials in the rare compared to the abundant presence of gap junctions! Whereas the propagation velocity increased by a factor of less than 2, the number of gap junctions increased more than a thousandfold. Hence, there is a poor correlation between gap junction number and electrical coupling. Furthermore, the increased propagation velocity could have been caused by factors other than increased gap junctions such as hormonally induced changes in membrane resistance. The increase in gap junction number is positively correlated with increase in spread of ^{3}H-2-deoxy-glucose [12], and hence may be associated with the need for synchronization of hormonal stimulation during labour.

Spread of electrotonic potentials

One can create a transient voltage change across the cell membrane by application of a shortlasting potential gradient across a pair of extracellular electrodes in many intestinal tissues. Such an 'electrotonic potential' spreads passively from cell to cell (fig. 5). The extent of the spread can be seen as a measure of electrical (electrotonic) coupling [26]. Indeed, it is the method employed to measure the so-called space constant which is used to indicate the degree of electrotonic coupling. The space constant is the distance over which a passively propagating potential loses 63% of its amplitude. Its value is roughly proportional to $\sqrt{\frac{r_m}{r_i}}$, where r_m is the transverse membrane resistance and r_i is the longitudinal cytoplasmic resistance. The longitudinal resistance also includes the intercellular communication pathway. Hence gap junctional conductance will markedly influence r_i. In the canine colon circular muscle, the space constant measured along the long axis of the circular muscle cells is 2.6 mm. Along the short axis of these cells, the space constant is 0.4 mm, explained by the notion that along the short axis the electrotonic potentials will have to encounter a larger number of cell-to-cell contacts [26].

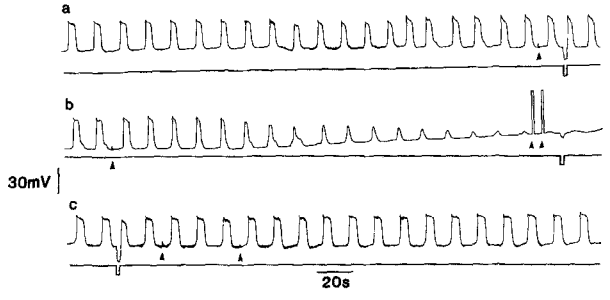


Figure 5. Electrical coupling in canine colonic smooth muscle. Spontaneous slow wave type action potentials are recorded from the submucosal surface of the circular muscle layer. An electrotonic potential generated by extracellular electrodes 1 mm from the recording electrode has propagated to the cell (pulse in tracing *a*). Heptanol (0.35 mM) causes a marked reduction in the amplitude of the electrotonic potential (pulse tracing *b*), presumably because of inhibition of cell coupling hence inhibition of electrotonic current spread. In the presence of heptanol injection of 1 nA current through the microelectrode produces a dramatic increase in voltage deflection since inhibition of cell coupling has increased the input resistance (2nd and 3rd arrow tracing *b*). Tracing *c* shows recovery from the effect of heptanol.

Electrotonic current spread, as discussed above, is abolished by heptanol or octanol in concentrations known to block gap junction conductance [29]. This is consistent with the hypothesis that conducting gap junctions are involved in electrotonic current spread. The relationship between gap junction number and extent of electrotonic coupling is unclear; space constants in tissues where gap junctions are rare (pre-term myometrium)[47] or abundant (submucosal surface of canine colon)[26] can be similar. There does not seem to be a positive correlation between gap junction number and electrotonic current spread. This was confirmed by Kannan and co-workers. In the canine trachealis [30] 4-aminopyridine was shown to increase gap junctions. The electrophysiological properties were studied later [31]; it was observed that the space constant was actually reduced from 2.16 mm to 1.75 mm. A reduction in electrotonic current spread associated with increased gap junctional coupling could suggest that increasing electrotonic coupling effectively increases three-dimensional current spread, thus reducing the space constant.

Measurement of input resistance

When one injects current into a single isolated cell through the recording electrode, the membrane potential shows a voltage deflection due to passage of current through the membrane; hence, an input resistance can be measured. When one injects current into a cell within a tissue that is well coupled by gap junctions, most of the injected current shunts through low resistance pathways (fig. 5). This low resistance could result in only a small increase in voltage across the membrane of the cell in which the current is injected, giving little or no recordable change of membrane potential [29, 46]. When one repeats this procedure in the presence of heptanol [29] (fig. 5) or octanol [8] input resistance becomes measurable or markedly increases. These alcohols inhibited cell coupling presumably through inhibition of gap junction conductance, although interference with other types of cell contacts have not been excluded. A comparative study is warranted of tissues with different structural types of cell coupling using the intracellular current injection technique as a means of measuring cell coupling.

Coupling in tissues where gap junctions cannot be recognized by electron microscopy

Gap junctions can be recognized by electron microscopy because there is aggregation of gap junction channels resulting in apparent fusion of plasma membranes seen as a seven-layer complex [55]. In some tissues gap junctions are not recognized by EM techniques.

The argument can be heard that one only needs one gap junction protein assembly between cells to have electrical coupling. Reported single channel conductances range from < 20 pS for β cells [40] to ~ 165 pS for cardiac

cells[57]. In one of a pair of pancreatic beta cells, a voltage pulse of 50 mV created a very small junctional current of 20 pA[40]. Whether the presence of such small gap junctions would provide enough current flow for propagation of action potentials in the three-dimensional intestinal musculature is questionable. In a cell pair, current received through a gap junction has no route through which to dissipate but the one coupled cell and may excite the membrane. In a syncytium the current may pass through other gap junctions into neighbouring cells. Whether cell coupling through tiny gap junctions would be more efficient than field coupling across a large area of closely apposed membranes has to be determined. It has been shown both theoretically and experimentally that an extracellular electric field generated by an action potential can drive excitatory current across the membrane of a neighbouring cell[49,53]. Electrical field coupling or ephaptic coupling needs close apposition membranes which are commonly observed. Certain three-dimensional structures are particularly suitable for this type of coupling: Bardakjian and co-workers (unpublished) showed that two model cells with a cytoplasmic process of one cell invaginating the surface of the other cell (ball-and-socket junctions) provide strong ephaptic coupling. These structures have been identified in intestinal smooth muscle by Gabella[17], and we have observed similar structures in the canine colon. Note that the cell-to-cell distance in the 'ball-and-socket' junctions is similar to (not smaller than) the distance between the apposed membranes in the interstitial cell network within the Auerbach's plexus of the small intestine (fig. 2).

Longitudinal muscle of the small intestine

No gap junctions have been reported to connect cells in the longitudinal muscle layer of the small intestine[15], nor has connexin 43 been detected[20]. Nevertheless, during phasic contractile activity, the muscle cells behave in a synchronized manner. Its mechanism of electrical communication is not resolved.

Zamir and Hanani[58] reported dye coupling within the longitudinal muscle of the small intestine of the guinea pig, where gap junctions are reportedly absent when assessed by electron microscopy. Up to ten cells were stained after injection in a single cell. Technical questions have to be raised related to this study; there is absence of a clear gradient in the dye staining, cells were stained not in direct contact with the cell injected, and there was no monitoring of the identity and health of the cells injected. Nevertheless, it appears that dye spread occurred in the longitudinal muscle. This would indicate the presence of a pathway connecting the intercellular compartments, hence a gap junction like structure, in the longitudinal muscle. Although no quantitative comparison was done, it was stated that dye spread was not different comparing longitudinal muscle and circular muscle, this would mean that the effectiveness of the intercellular communication pathway in the longitudinal muscle was similar to the electron-microscopically identified gap junctions in the circular muscle. This notion is supported by a study in the small intestine of the rabbit showing that electrotonic current spread was similar in longitudinal (space constant 0.97 mm) and circular (space constant of 1.02 mm) muscle[10a], however, Connor et al.[13a] observed different space constants in the cat small intestine: 3.5 mm for the circular muscle and 0.7 mm for the longitudinal muscle.

Arterial smooth muscle

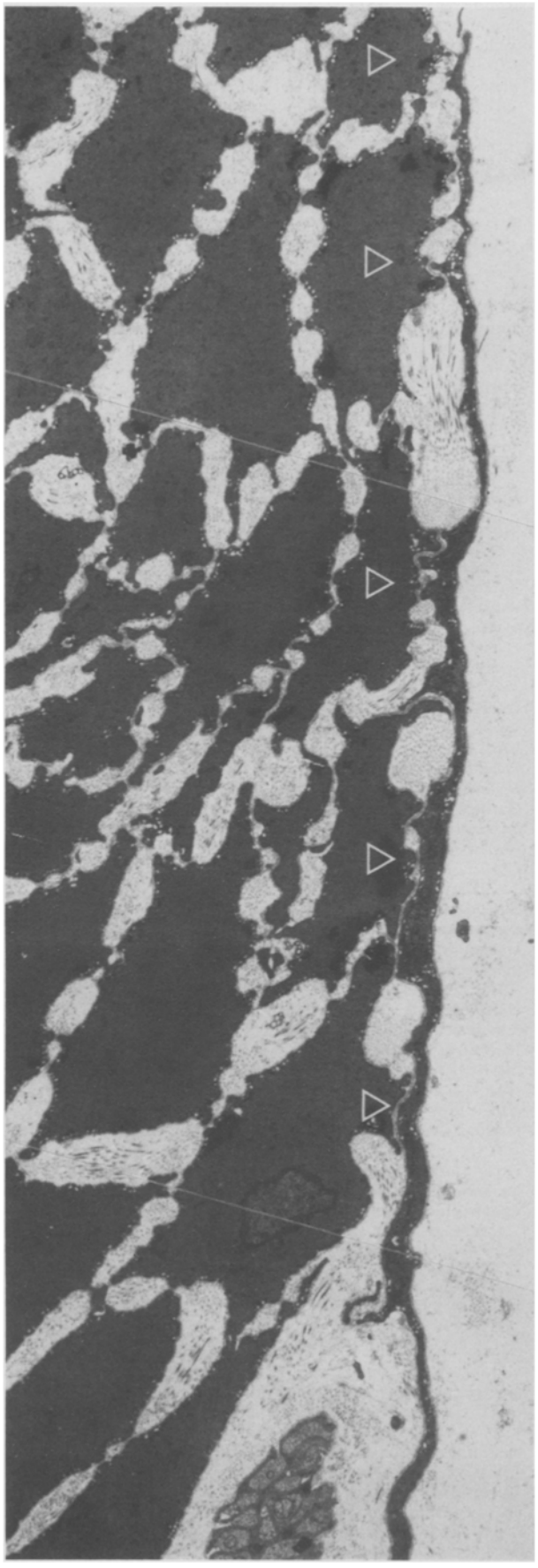
Smooth muscle cells of arteries are electrically coupled but gap junctions are rare or absent as detected by EM. In the pig coronary artery, although the absence of EM recognizable gap junctions was confirmed, staining by immunohistochemistry using an antibody against connexin 43 showed discrete punctation in some regions of some preparations[5]. Furthermore, after intracellular injection of lucifer yellow, dye spread was observed. It was concluded that arterial smooth muscle cells are connected by hydrophilic channels that are not likely aggregated into large plaques.

In summary, tissues that not possess electron-microscopically recognizable gap junctions but do show dye coupling may have gap junctions built up of connexin 43 protein not aggregated into plaques, or they may possess hydrophilic channels not characterized to date. Tissue that does not show dye coupling but does exhibit electrical coupling may possess hydrophilic channels impermeable to the dye, or its cells may not be metabolically coupled. Whether field coupling only becomes important in the absence of hydrophilic channels or occurs more generally, still has to be established.

Role of interstitial cells of Cajal in intercellular communication

Interstitial cells of Cajal (ICC) form a one-cell-layer-thick, three-dimensional network, which either covers the submucosal surface of the circular muscle as in the canine colon[6] or is housed in the myenteric plexus as in the small intestine[55,56]. Two major roles for ICC are proposed: 1) transmission of neural inputs to smooth muscle[6,7,16,25], and 2) involvement in providing pacemaker activity to intestinal smooth muscle[24,55].

It is intriguing that in the various networks of interstitial cells of Cajal, cell-to-cell coupling characteristics are quite distinct. In the circular muscle of the canine colon, it is the ICC network and associated smooth muscle cells at the submucosal border that have a very high gap junction density compared to the rest of the musculature as determined by immunoreactivity to connexin 43 antibody (Huizinga et al., unpublished). In the rabbit small intestine, two subsets of ICC occur, one associated with Auerbach's plexus, another one with the deep muscular plexus. ICC associated with the deep muscular plexus have numerous and large gap junctional contacts but very little 'close apposition' contacts (fig. 2). ICC associated with Auerbach's plexus have no gap junctional cou-



pling, but extensive 'close apposition' coupling (fig. 2). Most cardiac tissue is coupled by gap junctions, but gap junctions are rare in the core of the sino atrial node [10, 48]. In fact, low gap junction conductance or a higher input resistance is seen as beneficial for pacemaker cells since less current shunting will occur and less current will be needed to create voltage changes (i.e. pacemaker potentials). In the ICC network, cells connected by gap junctions to pacemaker cells could result in current sink. Hence, the primary function of the gap junctions may be the synchronization of metabolic activity hypothesized to trigger pacemaker activity [28]. A better understanding of the basis of metabolic regulation of the intestinal pacemaker activity and metabolic coupling of pacemaker cells is needed [28]. In addition, theoretical models using physiological parameters are essential for the development of concepts in this area.

An intriguing question with respect to electrical coupling of tissue is whether there is a role for specialized cells. What is the exact function of ICC in this respect? ICC can be observed in the submucosal border of the canine colon to connect up to 10 smooth muscle cells in longitudinal direction (fig. 6). Interstitial cells are seen to connect the serosal side with the myenteric plexus side of the longitudinal muscle (see fig. 18 in Thuneberg [56]); surely the presence of such cells inevitably provides a pathway for electrical communication between the two sides of the muscle layer. What is the physiological importance in cell communication of interstitial cells that structurally connect different parts of a tissue? Painstaking structural analysis of various parts of the gut is needed to provide us with more data.

A recently proposed role for ICC in the canine colon is that the network of ICC at the submucosal border of the circular muscle is the pathway through which longitudinal propagation, or synchronization, of active potentials across septa occurs (Liu and Huizinga, unpublished). The circular musculature is divided into discrete circumferentially oriented muscle bundles, the lamellae. The septa, dividing the lamellae, regularly extend all the way to the myenteric plexus. The ICC network extends as a complete network across the septa at the submucosal surface. We have shown that propagation across lamellae occurs through the ICC network. This hypothesis is consistent with data obtained in the cat colon [13].

Figure 6. Intercellular communication through interstitial cells of Cajal. Electron micrograph of canine proximal colon, magnification × 6800. The section contains cross sectioned circular muscle cells (left) and a large connective tissue septum (right). Five circular muscle cells are in contact with one very long process of an interstitial cell of Cajal. At this low magnification, the ICC can be seen to share two features with the smooth muscle cells: a large number of caveolae and a continuous basal lamina. A nerve fascicle of the submuscular plexus is seen at the bottom part of the micrograph.

Gap junctional communication between vascular smooth muscle and endothelial cells

It is now realized that normal regulation of blood flow is dependent on communication between vascular smooth muscle cells and endothelial cells, as illustrated by the important role of nitric oxide synthesized by endothelial cells, in vascular relaxation [1]. It was recently established that vascular smooth muscle cells, endothelial cells and pericytes are all capable of communicating intercellularly as assessed by dye transfer [32] and all use connexin 43 gap junction protein [32, 33]. The vascular system may be an ideal model for the study of metabolic coupling.

In summary, intercellular communication is essential for tissue function and its regulation may form an important physiological means of management of tissue function. Breakdown of communication as an underlying cause of pathophysiology is likely, but poorly understood and investigated. Many intriguing questions are awaiting answers, such as the role of interstitial cells in communication. The study of electrical coupling requires the use of various methods since different mechanisms may be employed physiologically to obtain passive and active propagation, or synchronization of electrical events. We urgently need more knowledge about mechanisms of cell coupling by means other than gap junctions; the effectiveness and role of ephaptic coupling have to be elucidated. There is no direct correlation between the density of gap junctions and either the degree of synchronization of electrical activity or the degree of electrotonic current spread. Hence, the role of gap junctions in functions other that electrical coupling has to be further investigated; they likely play a role in synchronization of hormonal stimulation in the myometrium during labour and may play a role in synchronization of metabolically activated pacemaker activity in the gut.

Acknowledgments. The Medical Research Council of Canada provided the funds for this study. Collaboration with the University of Copenhagen was made possible by a NATO grant, and a grant to L. Thuneberg from the Novo-Nordic Foundation, Copenhagen. We appreciate the electron microscopic assistance of Dr Irene Berezin.

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0014-4754/92/100932-10$1.50 + 0.20/0

Human slow wave sleep: A review and appraisal of recent findings, with implications for sleep functions, and psychiatric illness

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Abstract. Recent findings concerning human slow wave sleep (hSWS-stages 3 + 4; delta EEG activity) are critically reviewed. Areas covered include the significance of the first hSWS cycle; hSWS in extended sleep; relationship between hSWS, prior wakefulness and sleep loss; hSWS influence on sleep length; problems with hSWS deprivation; influence of the circadian rhythm; individual differences in hSWS, especially, age, gender and constitutional variables such as physical fitness and body composition. Transient increases in hSWS can be produced by increasing both the quality and quantity of prior wakefulness, with an underlying mechanism perhaps relating to the waking level of brain metabolism. Whilst there may also be thermoregulatory influences on hSWS, hypotheses that energy conservation and brain cooling are major roles for hSWS are debatable. hSWS seems to offer some form of cerebral recovery, with the prefrontal cortex being particularly implicated. The hSWS characteristics of certain forms of major psychiatric disorders may well endorse this prefrontal link.
Key words. Human sleep; slow wave sleep; sleep loss; circadian rhythm; brain metabolism; thermoregulation; schizophrenia; depression; frontal cortex.