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Cell-to-cell coupling assayed by means of electrical measurements

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Summary. The importance of electrical measurements in the evaluation of cell-to-cell coupling in heart muscle was discussed. The presence of gap junctions in heart and smooth muscle, and the implications of this for electrical synchronization and healing-over, was emphasized. Moreover, the modulation of junctional resistance by Ca, protons and cAMP was reviewed.
Key words. Cell coupling; heart; electrical.

Nur allein der Mensch
Vermag das Unmögliche,
Er unterscheidet,
Wählet und richtet;
Er kann dem Augenblick
Dauer verleihen.
Goethe

Intercellular coupling through low resistance junctions represents a very old mechanism of cell-to-cell communication. In simple organisms such as sponges and medusae without nervous tissue, the epithelial cells receive external stimuli and convert them into electrical pulses which are conducted in all directions through low resistance junctions[31]. Evidence exists that in excitable tissues the intercellular junctions are essential for the spread of electrical activity and for electrical synchronization. It is my intention in this manuscript to review how electrical measurements have contributed to the concept that cells are in communication. In 1954, Sjöstrand and Andersson[37] demonstrated that cardiac muscle is composed of individual cells surrounded by clearly discernible membranes. As the electrical impulse propagates through cardiac muscle as though it consists of one large cell the question is, how can a cardiac myocyte initiate electrical activity in neighboring cells?

A possible explanation is the release of some transmitter

which depolarizes the cell membrane of surrounding myocytes. It is known from the chemical theory of synaptic transmission that a chemical machinery is necessary for the synthesis and storage of transmitters into synaptic vesicles. There is no morphological or biochemical evidence for chemical transmission between cardiac myocytes.

The other possibility is that the electrical impulse propagates through local circuit flow and that internal current flows between neighboring cells via low resistance junctions.

Evidence has accumulated that heart tissues have cable properties[43,44]. The first conclusive evidence that heart cells are electrically in communication was presented by Weidmann, 1952[43]. When current is injected into a canine Purkinje cell appreciable changes in membrane potential can be seen in adjacent cells. The core resistivity is quite low and the space constant (1.9 mm) large in comparison with the length of a single Purkinje cell (125 μm).

In 1965, Barr et al.[2] demonstrated that impulse conduction in atrial muscle is blocked by increasing the extracellular resistance in the central portion of a thin bundle. The blockage of the action potential can be reversed by connecting an appropriate resistance between the solutions located on the left and right sides of the central gap. These findings certainly demonstrated that low external and internal resistances are necessary for the propagation of the impulse.

But are intercellular junctions indeed necessary for the propagation of the impulse? Considering that cardiac fibers are composed of cells with a surface cell membrane with similar electrical properties and separated by a gap of ~ 200 Å at the intercalated disc, the propagation of the electrical impulse would be feasible if it jumps the gap and stimulates the next cell.

It is known that passive electrical properties can jump across a quite short inexcitable zone. If the signal amplitude is not reduced to less than 1/5, the threshold will be achieved in the cells beyond the block and consequently propagated action potentials will be produced. As the terminal membrane impedance falls with the inverse square of the fiber diameter it is not surprising that in giant septate axons the 'attenuation' of the electrical impulse across the septa is about 1/10 and transmission along the axon is almost possible, even assuming the absence of intercellular channels[30]. For fibers of small diameter (5 μm), however, the situation is quite different and the impulse will not be able to jump a 150 Å gap because of a large attenuation factor[30]. It seems, then, possible to conclude that in heart fibers with small diameter, the electrical impulse will not be able to jump the gap (150–200 Å) located at the intercalated discs and the presence of intercellular channels is, indeed, required for impulse propagation.

When current is applied through extracellular electrodes to bundles from mammalian heart the space constant varies from 800 μm[45] to 1300 μm[29], values which are large compared to the length of a single cell (125 μm). These findings support the view that cells are electrically coupled.

An appreciable decrement of the electrotonic potential is described in rat atrium[49] when current is injected intracellularly. In this particular case, because of the multidimensional spread of current, the decrease in voltage with distance is not exponential[50], but the value of the space constant varies from 50 to 400 μm and certainly larger than the length of a single atrial cell[50].

Barr et al.[2] showed that the nexus is most probably the site of intercellular communication, because when a hypertonic sucrose solution is used the impulse conduction is blocked concurrently with the rupture of the nexuses. Similar results were obtained in toad heart by De Mello et al.[21].

In practically all systems so far studied, the electrical coupling between cells is associated with the presence of gap junctions[16]. In cultured heart cells, for instance, the coupling resistance is quite high (> 100 MΩ) at the moment of cell contact but starts falling immediately after reaching values of 20 MΩ at the moment of electrical synchronization[6]. In this case, the formation of a few interchannels is enough to establish the electrical coupling[47]. It is important to emphasize that in some tissues showing electrical coupling conventional electromicroscopic studies fail to show gap junctions. However, when freeze-fracture studies are performed, organized junctions or single scattered particles containing intercellular channels have been identified.

The determination of cell-to-cell coupling by electrical measurements offers some difficulties. When electrical current is injected into the cell the change in membrane potential recorded from a neighboring myocyte is dependent on the amount of current injected, the amount leaked through the non-junctional cell membrane of the injected cell, the myoplasmic resistivity, the junctional resistance and finally the non-junctional resistance of the non-injected cell.

Changes in non-junctional membrane resistance can, indeed, alter the coupling coefficient (V_2/V_1)[42]. It is recommended that the time constant of the cell membrane be measured concurrently with the coupling coefficient in order to rule out any possible influence of the non-junctional membrane resistance on the interpretation of the results.

Measurements of the input resistance are also useful because an increase in resistance without a change in non-junctional membrane conductance is probably due to an increase in junctional resistance. It is interesting to add that if current pulses are injected into a cell of a 3-dimensional syncytium and the voltage changes are recorded from the same cell, an increase in input resistance 'only' means an increase in myoplasmic resistance or an enhanced cell-to-cell coupling[4].

In many systems the junctional resistance is constant[3]. Although the resistivity of the junctional membrane is very low some uncertainty exists in the determination of the junctional area. Values of 1 ohm·cm^2 have been reported for cardiac muscle[44] and for Chironomus salivary gland[34].

The junctional capacity is probably similar to that found in other membranes (1 μF/cm^2). Freygang and Trautwein[25], however, found a time constant of 64 μs in the longitudinal circuit of Purkinje fibers which they ascribed to capacitative coupling across the intercalated disc. A value of 370 μF/cm^2 was also found in elements oriented longitudinally along the frog ventricle[5]. The precise meaning of these findings is not known and further studies are necessary.

In trabeculae dissected from mammalian cardiac muscle the density of gap junctions seems to be higher in the longitudinal direction. Using ventricular trabeculae of the sheep Clerc[7] found the transverse intracellular resistance (r_i) to be 9.4 times the longitudinal r_i. Moreover, the conduction velocity in the transverse direction was 3 times lower than the values found for longitudinal propagation[7].

Indeed, variations in number and size of gap junctions seem to represent an important mechanism of regulation of cell-to-cell communication. Although it is known that the synthesis and destruction of gap junctions occurs before and during the differentiation process[27], no information is available about whether dynamic change of intercellular channels occurs in adult cardiac muscle. It is known, however, that the density and size of these junctions varies among the tissues of the heart. In the sinoatrial node and atrioventricular node of adult rabbit, for instance, the density of gap junctions is much smaller than that found in the atrium[32].

Despite the small size of gap junctions, the cells are electrically coupled[13,15]. The small number of intercytoplasmic channels in the A–V node seems to be in part responsible for the small space constant (0.4 mm) found in this tissue[13].

Changes in junctional conductance can be also achieved in adult cardiac tissues, as well as in other tissues, by altering the intracellular concentration of Ca or by reducing the pH_i[16,41,38]. When Ca ions are injected iontophoretically in-

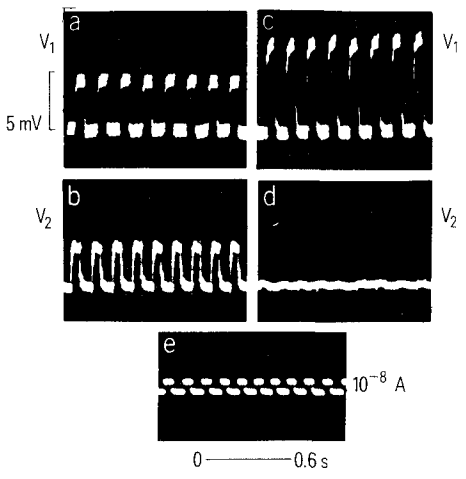

Figure 1. Typical effect of intracellular Ca injection on the electrical coupling of canine Purkinje cells. *a* and *b* show V_1 and V_2 in control; *c* and *d* after 410 s of Ca injection showing cell decoupling; outward current pulses (60 ms duration, 5 Hz, 10^{-8} Å). (From De Mello[16] with permission).

side canine Purkinje cells the electrical coupling is gradually reduced and total cell decoupling is produced[11] (fig. 1). Such a decrease in electrical coupling cannot be ascribed to a great fall in surface membrane resistance because the input resistance of the injected cell was increased[11]. Similar results were found in other structures[35].

The question whether Ca is a physiological modulator of junctional conductance is not clear. Certainly, the Ca concentration ($5 - 8 \times 10^{-5}$ M) necessary to abolish the electrical coupling in Chironomus salivary gland is high. Moreover, the high buffer capacity of the cytoplasm for Ca and the low diffusion of the ion in the sarcoplasm make it difficult to visualize how Ca can modulate gj under physiological conditions. Considering, however, that modulation can be

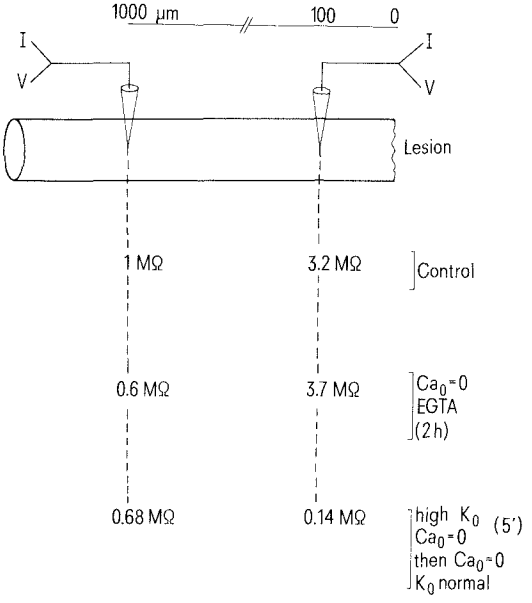

Figure 2. Healing-over of guinea pig taenia coli achieved in normal Tyrode solution and Ca-free EGTA solution, but suppressed by exposing the tissue to high K (60 mM) solution, Ca-free solution for 5 min and then equilibrating in Ca-free K_o normal. Numbers indicate values of input resistance recorded with a single microelectrode (used to inject current and record the voltage changes) impaled near the lesion and in the middle of the fiber 25 min after damage (De Mello, unpublished).

achieved with small changes in concentration, and the compartmentalization of the cell interior, further studies are necessary to rule out the role of Ca as a physiological modulator of gj. There is no doubt, however, that Ca is a good cell 'decoupler' under pathological conditions. Indeed, when heart cells are damaged a high-resistance barrier is quickly established near the injury (healing-over[22]) isolating the lesioned cells from the adjacent normal myocytes. In Ca-free solution no healing-over is accomplished[8, 21]. The sealing process is of fundamental importance for the preservation of heart function after injury. Lack of healing-over would permit the spread of injury currents throughout the normal tissue with consequent depolarization. Fortunately, as stressed by Engelmann 'der Tod schreitet nicht von Zelle auf Zelle fort'[22].*

The drastic increase in junctional resistance caused by Ca or by extremely low pH (5.5 or lower)[17] is considered to be the mechanism by which healing-over is produced. So, it is not surprising that in skeletal muscle no healing-over can be seen[10, 36].

In many tissues without intercellular junctions cell injury is followed by a flow of protoplasm through the damaged area which is soon halted by the formation of a new membrane which insulates the droplet of cytoplasm from the extracellular fluid[28]. This new membrane, however, is not able to avoid the depolarization of the skeletal muscle fiber[10].

In tissues containing intercellular junctions healing-over is easily achieved. In somatic musculature of *Ascaris*, for instance, in which the somatic muscle cells are coupled through low resistance junctions, sealing is quickly established[9]. The same phenomenon exists in liver cells (De Mello, unpublished) in which 1.5% of the membrane consists of gap junctions.

Recently, we investigated the phenomenon of healing-over in the intestinal longitudinal smooth muscle. It is known that guinea pig taenia coli has nearly perfect cable-like properties[39]. The space constant in this tissue is about 1.5 mm[39], a value much larger than the length of a single muscle cell. Moreover, the contribution of the junctional resistance in this tissue is of the same order of magnitude as the myoplasmic resistance[1].

Although in taenia coli gap junctions seem to be small (probably a few junctional particles[26]), the cells are electrically coupled. When the teania coli is damaged, healing-over is produced within 1–2 min. Not only the depolarization produced by lesion is quickly reversed, but the input resistance increases towards the cut-end (fig. 2), supporting the view that a high resistance barrier is established near the lesion[23]. It is interesting to add that in taenia coli healing-over is produced even in preparations exposed for 2 h to Ca-free plus EGTA (2mM) solution. Although the removal of external Ca seems not to be important for healing-over, the depletion of Ca stores achieved by exposing the tissue briefly to $LaCl_3$ (2 mM) plus Ca-free solution or high K solution, free of Ca, abolishes the sealing process[23]. This finding might indicate that in this muscle intracellular Ca stores represent an important source of the ion for sealing.

Cyclic-AMP; a physiological modulator of junctional resistance

It is well known that Ca^{2+} and cyclic nucleotides are intimately related in the control of cell function induced by many hormones[33]. The free $\{Ca^{2+}\}_i$ is, in part, regulated by the concentration of cAMP while a rise in free $\{Ca^{2+}\}_i$ reduces the concentration of cAMP probably by inactivation of adenylate cyclase[18].

It seems then reasonable to ask whether cyclic nucleotides are involved in the control of junctional conductance. Evidence is now available that this is the case. When cAMP is

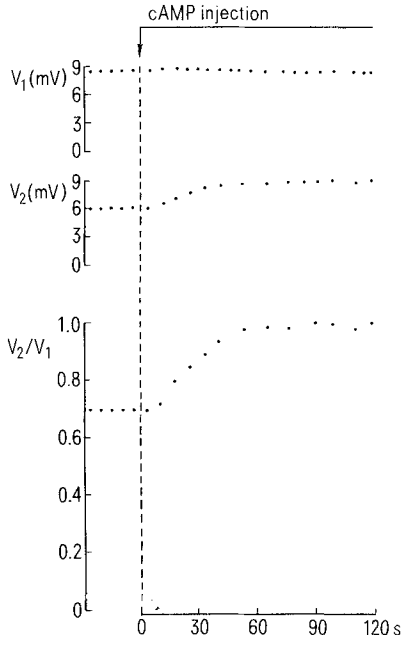

Figure 3. Effect of intracellular injection of cAMP on the electrical coupling of canine Purkinje cells. As can be seen, the coupling coefficient (V_2/V_1) was increased within 30 s reaching a plateau in about 60 s. Temperature −37 °C. (From De Mello[19] with permission).

electrophoretically injected into a cardiac cell the coupling coefficient (V_2/V_1) is increased within 30 s (see fig. 3) reaching a plateau in about 60 s. The effect of the nucleotide is quickly reversed when the release of cAMP into the cytosol is interrupted.

It is not known, as yet, how cAMP increases the electrical coupling. An increase in non-junctional membrane resistance can be ruled out because the time constant of the cell membrane is slightly reduced by the intracellular injection of the compound[19]. A decline in free $\{Ca^{2+}\}_i$ also seems unlikely because the injection of the compound was made in quiescent fibers, where the free $\{Ca^{2+}\}_i$ is very low (10^{-7} M), and an increase in pH_i is not probable because the Na salt of cyclic AMP had a neutral pH. The possibility that the compound activates a kinase located at the gap junction, with consequent phosphorylation of gap-junctional proteins and increase in channel permeability, has been proposed[18]. The finding that cAMP injection increases the coupling coefficient within 30 s seems to indicate that the nucleotide has a direct effect on gap-junctional molecules.

In mammalian fibroblasts in culture exposed to cyclic AMP or dibutyryl-cAMP plus caffeine, the cell-to-cell transfer of a series of fluorescent compounds starts to increase in about 1 h, reaching a maximum by 4 h[24], a result probably due to an increase in the number of gap junctional membrane particles. It is not known, however, whether in other tissues exposed chronically to dB-cyclic AMP the number of junctional particles is also increased. Assuming that this is the case, then it is possible to think that hormones such as epinephrine that increase the intracellular concentration of cAMP can induce: a) a quick increase in junctional conductance, probably by acting directly on gap junctional molecules; b) an increase in the synthesis of gap junctional particles in the long run.

The increase in the electrical coupling of cardiac cells produced by epinephrine, for instance, is seen within seconds[20]. It is important to emphasize that the increment in coupling coefficient caused by epinephrine during diastolic depolarization is not due only to a decline in r_i but also to an increase in r_m[20].

The gradual increase in coupling seen during diastolic depolarization in Purkinje fibers not exposed to the drug has an important physiological implication, the gradual increase in electrical synchronization during diastole. If this mechanism exists in the sinoatrial node then the gradual increase in electrical coupling during diastole generates an appreciable functional unit which represents the synchronized firing of many pacemaker cells with a much larger excitation power than isolated cells[20].

It seems, then, likely that the physiological role of cAMP in the control of cell-to-cell communication in heart is mainly related to a quick increase in gj like that found during stress, when an increase in heart rate, strength of contraction and velocity of impulse conduction is seen in response to epinephrine release.

It is not known, however, whether the number of intercellular channels is increased in heart muscle under pathological conditions such as an increase in sympathetic tonus, or pheochromocytoma, in which the heart tissues are chronically exposed to high plasma levels of catecholamines.

Physiological and pathological implications of the electrical coupling

The presence of intercellular channels between cardiac cells and the variation of junctional conductance and permeability brought about by intracellular factors[16] have many physiological and pathological implications.

The conduction velocity (ϕ), for instance, is influenced by the intracellular longitudinal resistance (r_i) according to the following equation:

$$\phi 2 = \frac{1}{\tau_{foot}\, r_i\, cm}$$

During the propagation of an impulse, action currents flow in local circuits from the depolarized region along the inside of the fiber; that is, along the myoplasm and the intercellular junctions, outward through the adjacent resting myocytes, back along the external fluid and inward through the depolarized membrane, closing the circuit. It is then easy to visualize that the current flowing along the inside of the fiber is impaired by a decrease in junctional conductance. Not only is the conduction velocity reduced, but less current will be available to depolarize the cells located far away from the site of stimulation.

Hypoxia or metabolic inhibitors enhance the intracellular longitudinal resistance due to a fall in ATP concentration and consequent rise in free $\{Ca^{2+}\}_i$, and a decrease in pH_i[14,48]. Inhibition of the Na/K pump produced by digitalis also increases r_i[46] through the activation of Na/Ca exchange and increase in free $\{Ca^{2+}\}_i$[12]. The rise in r_i is, in part, responsible for the block of impulse conduction seen in the A–V node exposed to high doses of digitalis. The presence of a high intracellular resistance in the A–V node[13] certainly increases the probability of A–V block in presence of digitalis.

Considering the evidence that in A–V and sinoatrial nodes an inward Ca current is responsible for the generation of the action potential, it is reasonable to think that in these tissues the maintenance of a low free $\{Ca^{2+}\}_i$ is even more important to avoid cell decoupling than in atrial and ventricular fibers. The finding that the permeability of gap junctions can be changed by Ca and H ions, as well as by cAMP, provides new avenues leading to a better understanding of heart physiology and pathology.

Cell decoupling can also be produced by a drastic fall in the resistance of the non-junctional membrane. When the sinoatrial node or the A–V node are exposed to acetylcholine, for instance, the electrical coupling is quickly abolished[13]. In the A–V node the space constant is, indeed, greatly reduced by ACh. Considering, however, the decrease in the time con-

stant of the cell membrane produced by ACh it seems likely that the suppression of cell-to-cell communication in this case is mainly due to a fall in surface membrane resistance[13]. Considering that ACh hyperpolarizes, and inhibits the pacemaker activity, the persistence of open channels between cells makes possible the spread of inhibition.

Thanks to the healing-over process a large number of heart cells are protected from being depolarized by the flow of injury currents. This important phenomenon is possible only because the heart cells are connected through channels that are closed immediately after lesion.

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