Action Potential Transfer in Cell Pairs Isolated From Adult Rat and Guinea Pig Ventricles

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An enzymatic procedure was used to obtain ventricular cells from adult rat and guinea pig hearts. Isolated pairs of cells were selected to study the action potential transfer from cell to cell and determine the resistance of the nexal membrane, $r_m$. For this purpose, each cell of a cell pair was connected to a patch pipette so as to enable whole-cell, tight-seal recording. Normal impulse transmission was observed when $r_m$ ranged from 5–265 MΩ. In these cases, the action potential in both cells occurred virtually simultaneously. An occasional failure in action potential transfer was seen in cell pairs whose $r_m$ had increased to 155–375 MΩ. In these cases, the impulse transfer across the nexal membrane occurred with considerable delay. Impulse transfer was completely blocked once $r_m$ was larger than 780 MΩ. Assuming a single connexon conductance of 100 pS, this would mean that more than 13 connexons are necessary to allow impulse transfer from cell to cell. Two single myocytes, gently pushed together, neither showed electrotonic interaction nor impulse transfer, thus rendering unlikely the possibility of an ephaptic signal transmission. (Circulation Research 1988;63:72-80)

In cardiac tissue, impulse propagation is determined by two sets of parameters: source factors and sink factors. The source factors involve excitability of the sarcolemmal membrane brought about via voltage- and time-dependent inward currents; the sink factors include passive electrical properties and the topology of cell arrangements (e.g., see Fozzard). A change in each of these parameters is expected to modify the conduction velocity in the heart. In fact, the influence of membrane excitability on conduction velocity has long been recognized, while the importance of intercellular connections was realized more recently. Interest in intercellular connections increased after it was established that the functional cell-to-cell coupling does not remain constant but can be modified under certain conditions (for review, see De Mello; see also Spray and Bennett)

In the past, studies focusing on impulse propagation were carried out on intact hearts, preparations dissected from different regions of the heart, or synthetic strands of cardiac cells. More recently, the introduction of enzymatic procedures for dissociating cardiac tissue (e.g., see Trube) enabled a novel approach. Besides single cells, these methods also yield functionally intact cell pairs (e.g., see Kameyama and Metzger and Weingart). Such cell pairs enable impulse transmission to be investigated at the cellular level for the first time.

This article describes experiments performed on pairs of cells isolated from adult rat and guinea pig ventricles. The experimental approach adopted involved current- and voltage-clamp measurements. It allowed simultaneous study of action potential transfer from cell to cell and the nexal membrane resistance ($r_m$) limiting the intercellular current flow. Preliminary data on this topic have been published before.

Materials and Methods

Cell Preparation

Ventricular cell pairs were obtained by means of enzymatic dispersion of adult rat or guinea pig hearts. The isolation procedures employed have been described before in detail. For rat hearts, it involved the use of collagenase (type I, code CLS 4194, Worthington, Freehold, New Jersey); for guinea pig hearts, a combination of collagenase (type I, code C0130, Sigma Chemical, St. Louis, Missouri) and hyaluronidase (type I-S, code H3506, Sigma). All experiments were carried out at room temperature (22°C) in the presence of the following bathing solution (mM): NaCl 150, KCl 4, CaCl2 5,
MgCl₂ 1, HEPES 5 (pH 7.4), glucose 5, and pyruvate 2.

**Experimental Setup and Patch Pipettes**

The experiments were carried out in a chamber consisting of a Perspex frame with an attached glass bottom (volume, 1 ml). The chamber was mounted on the stage of an inverted microscope equipped with phase contrast optics (Diaphot-TMD, Nikon; Nippon Kogaku, Tokyo, Japan). A TV system (JVC; Victor Company, Tokyo, Japan) facilitated the visual control of the cells and pipettes. Patch pipettes were pulled from microhematocrit tubing (code 1021; Clay Adams, Parsippany, New Jersey) using a micro-processor controlled puller (model BB-CH, Mécanex, Geneva, Switzerland). Fire-polished pipettes (tip size, approximately 2 μm) were filled with the following solution (mM): NaCl 10, potassium aspartate 120, KCl 10, CaCl₂ 1, MgCl₂ 1, EGTA 10, and HEPES 5 (pH 7.2). In some experiments, potassium chloride or cesium chloride was used instead of potassium aspartate.

The equipment employed to manipulate the pipettes and to measure and record the electrical signals has been described previously. The settling time of the custom-built amplifiers was approximately 0.1 μsec. Control experiments (two patch pipettes fixed to a single cell) revealed that the membrane potential was adequately controlled within 1 msec. Series resistances arising from the pipette tips (direct current resistances: 1.5–2.5 MΩ and 0.8–1.3 MΩ, for aspartate and Cl⁻ containing solutions, respectively) were compensated for previous to an experiment. No corrections were made for access resistances arising from membrane fragments inside the pipette tip. Previous studies have shown that this does not severely affect the results. Membrane potentials were corrected for liquid junction potentials between the pipette filling solution and the bath solution (Cl⁻ containing solutions, −2 mV; aspartate containing solution, −10 mV).

**Signal Recording and Data Analysis**

During an experiment, each cell of a cell pair (cell 1, cell 2) was attached to a patch pipette so as to enable whole-cell, tight-seal recording. The pipettes were in turn connected to separate amplifiers that allowed operation in the current- or voltage-clamp mode (see Figure 1A). The current-clamp mode was used to inject rectangular current pulses and record the subsequent action potentials. The voltage-clamp mode was used to determine the nexal membrane resistance, rᵣ. In essence, this approach enabled the potential of the sarcolemmal membrane of each cell to be controlled (Vₑ,₁, Vₑ,₂) and thereby the voltage gradient across the nexal membrane (Vₚ) to be set, while monitoring the associated currents flowing through each pipette (Iₑ,₁, Iₑ,₂). Thus, when a voltage pulse was delivered to cell 1 (Vₑ₁), while cell 2 was kept at the common holding potential (Vₑ₂), Vₑ reflected the Vₑ and Iₑ corresponded to the current flowing across the nexal membrane (see Figure 1B). From these two quantities, that is, Vₑ and Iₑ, rₑ was determined.

**Results**

The experiments were performed on two-cell preparations in the side-to-side configuration. As a standard procedure, each cell pair was subjected sequentially to either of two protocols. In the one case, action potentials were elicited and recorded through use of the current-clamp mode. This involves application of depolarizing current pulses to one of the cells and recording of the elicited action potentials from the stimulated cell and the follower cell. In the other case, transmembrane currents were determined using the voltage-clamp mode. This includes establishment of desired transnexal voltage gradients and measurement of the associated junctional membrane currents. Alternate application of the two protocols allowed exploration of the relation between intercellular coupling and impulse propagation.

**Normally Coupled Cell Pairs**

The electrical behavior of an isolated cell pair under control conditions is documented in Figure 2. Figure 2A shows the standard signals obtained in the current-clamp mode. Both cells exhibited the same resting potential, that is, −85 mV, indicative of electrical coupling via intact nexal membrane. In such preparations, it is anticipated that differences in intrinsic membrane potential are masked by compensatory currents flowing from cell to cell. Depolarizing current pulses, constant in duration (20 msec) and frequency (0.5 Hz), but variable in amplitude, were applied to cell 1. As indicated,
current pulses of just threshold amplitude elicited an action potential in cell 1 (V1) and cell 2 (V2). It is tempting to assume that the action potential was transferred from cell 1 to cell 2 via current flow across the nexal membrane.

Figure 2B illustrates the standard signals recorded after switching to the voltage-clamp mode. Initially, the membrane potential of both cells was clamped to a Vm of -52 mV. Thereafter, a depolarizing clamp pulse (amplitude, +41 mV; duration, 200 msec; frequency, 0.5 Hz) was applied to cell 1, while the membrane potential of cell 2 was maintained. This gave rise to the current signals I1 and I2. I1 reflects the sum of two current components, I_m,1 and I_n. I_m,1 flows across the nonjunctional membrane of cell 1 and corresponds to the spontaneously decaying Ca^{2+} inward current, I_m (e.g., see Irisawa and Kokubun20), whereas I_n flows through the nexal membrane located between cell 1 and cell 2. The capacitative spikes in I_m, inwardly and outwardly oriented, were caused by the rapid "on" and "off" of the voltage pulse, respectively. I_n corresponds to I_n. The ratio V/v/I_n, defining r_n, turned out to be 41 MΩ.

Figure 3 repeats selected records from Figure 2A. To emphasize the temporal relation, the signals were superimposed and displayed on an expanded time scale. Trace I1 reproduces the current pulse injected into cell 1, while V1 and V2 correspond to the voltages measured from cell 1 and cell 2, respectively. Current injection gave rise to electrotonic responses that were similar in time course and magnitude. The depolarizations eventually reached threshold so that each cell fired an action potential. Close inspection of the fast-rising phase reveals no measurable delay between the two action potentials. This suggests that the two events occurred virtually simultaneously.

Immediately after the onset of electrical measurements this cell pair exhibited an r_n of 9 MΩ. The records presented in Figures 2 and 3 were obtained 12 minutes later, when r_n had increased spontaneously to 41 MΩ. The increase in r_n presumably was caused by elevation in [Ca^{2+}], (e.g., see Maurer and Weingart17) and/or elution of cytosolic compounds involved in connexon regulation. Of importance is that up to this stage, there was no indication of a delay in action potential transfer from cell to cell.

**Moderately Uncoupled Cell Pairs**

Figure 4 illustrates the performance of a cell pair with impaired impulse propagation. Figure 4A depicts current-clamp records displayed at compressed time scale. Both cells showed unstable resting potentials ranging from -71 mV to -77 mV. The spontaneous changes in V_m occurred synchronously in cell 1 and cell 2, suggesting that the cells are still coupled electrically. Injection of small current pulses into cell 1 (I_1; duration, 200 msec; frequency, 0.5 Hz) provoked subthreshold depolarizations in both cells. V1 and V2 differed considerably in amplitude, signaling the presence of a substantial voltage drop across the nexal membrane. Application of larger stimuli to cell 1 evoked action potentials in both cells in most trials. On two occasions, however, cell 2 failed to respond with an action potential. This indicates block of impulse propagation across the junction. In these cases,
current injection evoked an action potential in the stimulated cell (cell 1) and an electrotonic response in the follower cell (cell 2).

Figure 4B illustrates a successful action potential transfer at faster time resolution. Current injection into cell 1 was accompanied by a large electrotonic response in cell 1 and a small one in cell 2. The former eventually reached threshold, the latter remained subthreshold throughout. Despite this difference, both cells fired an action potential toward the end of the current pulse. This pattern of phenomena implies that the two action potentials are of different origin. In cell 1 it is a consequence of direct stimulation, whereas in cell 2 it reflects indirect stimulation via nexal current flow. Thus, this experimental situation unambiguously documents impulse propagation across the junctional membrane.

Figure 4C illustrates the voltage-clamp records used to determine the nexal membrane resistance prevailing under these circumstances. Starting at a \( V_H \) of \(-69 \text{ mV}\), a hyperpolarizing pulse (amplitude, \(-20 \text{ mV}\); duration, \(200 \text{ msec}\)) was applied to cell 1 (\( V_1 \)), while the membrane potential of cell 2 (\( V_2 \)) was maintained. This pulse protocol elicited the current signals \( I_1 \) and \( I_2 \). Again, \( I_1 \) represents the sum of two current components, \( I_{in,1} \) and \( I_o \). Under the prevailing conditions, \( I_{in,1} \) corresponds to the \( K^+ \) inward rectifier current (e.g., see Sakmann and Trube\(^{21}\)). \( I_2 \) represents a single current component, \( I_n \). Analysis of the junctional current signal \( I_j \) revealed an \( r_m \) of \(315 \text{ M\Omega} \), a value 60–80 times above control.

Figure 5 illustrates an experiment in which impulse transfer from cell to cell was blocked completely. By guest on April 13, 2017 http://circres.ahajournals.org/ Downloaded from
Correlation Between Action Potential Transfer and \( r_n \)

Table 1 summarizes the collected data from 30 different cell pairs. It documents the correlation between the state of impulse transfer (first column) and the \( r_n \) (second column) or the putative number of connexons involved (third column). The single connexon conductance was assumed to be 100 pS (see "Discussion"). In cell pairs exhibiting \( r_n \)s of up to 265 MΩ, no irregularities were observed with regard to action potential transfer. The critical range of sporadic failures in action potential propagation was associated with \( r_n \) values from 155 to 375 MΩ. Complete block of impulse propagation was found with \( r_n \) values beyond 780 MΩ. Table 1 shows that the measurements from different preparations varied substantially. Each type of impulse propagation covers a large range of \( r_n \) values. Part of it could be caused by variations in relative cell size and/or excitability of the sarcolemma. In this study, we have made no attempts to explore these possibilities.

In some cases of successful impulse transfer, we explored the possibility of a delay between the action potentials fired by the individual cells of a cell pair (e.g., see Figures 3 and 5). These experiments revealed no indication of a discrete impulse propagation across the nexal membrane for \( r_n \) values of 5–100 MΩ.

Current Flow Between Two Isolated Myocytes

The question arises whether or not two myocytes, when brought into physical contact, show impulse transfer from cell to cell. To investigate this possibility, the patch pipettes were connected to two myocytes clearly separated from each other. Subsequently, gentle agitation of both micromanipulators allowed the cells to be maneuvered close together and thus to establish an intimate side-to-side contact.

Figure 7 illustrates the result of such an experiment. Figure 7A shows the associated current-clamp records. Depolarizing current pulses applied to cell 1 evoked action potentials in this cell but not in the other one. Figure 7B shows the records obtained after switching to the voltage-clamp mode. Starting from a \( V_H \) of −65 mV, a strong depolarizing voltage pulse (amplitude, +41 mV; duration, 200 msec) was applied to cell 1. This gave rise to a large inward current in cell 1, followed by a time-dependent decay. In addition, it led to a small nexal...
One page of the document contains text discussing impulse transfer and nexal resistance in cell pairs. The text starts with a discussion of the simplest preparation appropriate for studying impulse transfer from cell to cell, mentioning cell pairs used in the present study. It notes that the equipment was not designed to study single channel properties, and the possibility cannot be ruled out completely.

**Discussion**

In the present study, experiments were performed that explored the transfer of the cardiac impulse at the cellular level. Cell pairs were used that had been isolated from adult guinea pig ventricular cells by means of an enzymatic method. As previously shown, such cell pairs exhibit normal electrical and diffusional coupling. They are the simplest preparation appropriate for a study of impulse transfer from cell to cell.

**Impulse Transfer and Nexal Resistance**

Regular impulse propagation was found in the presence of \( r_n \) ranging from 5 to 265 M\( \Omega \) (see Table 1, second column). If we assume a single connexon conductance of 100 pS (adult guinea pig heart cells, 100 pS [P. Maurer and R. Weingart, unpublished observations]; neonatal rat heart cells, 33–50 pS\(^{25}\); embryonic chicken heart cells, 165 pS\(^{26}\); adult rat lacrimal cells, 90 pS\(^{27}\)), this suggests that at least 38 connexons arranged in parallel are required to enable normal impulse transfer (see Table 1, third column). The first signs of disturbances in impulse propagation were observed in cases where \( r_n \) had increased to 155–375 M\( \Omega \); this would correspond to 25–65 connexons in operation. Complete block of impulse propagation was observed when \( r_n \) had reached values larger than 780 M\( \Omega \). From this finding it is inferred that at least 13 connexons are required to achieve a transfer of the cardiac action potential.

In coupled cell pairs, as in functional syncytia, the electrotonic voltage response of the follower cell depends on both the nexal membrane current generated by the leader cell and the electrical load on the leader cell. Therefore, the ratio of junctional resistance to input resistance (\( r_J/R_{in} \)) represents a useful parameter for expressing the uncoupling data. If we assume an \( R_{in} \) of 50 M\( \Omega \), \( r_J/R_{in} \) is then 0.1–5 in the case of regular impulse transfer, 3–7.5 in the case of occasional transfer failure, and 1 in the case of complete block. DeHaan and coworkers\(^{28–30} \) explored the synchronization of action potentials between embryonic heart cell aggregates exhibiting different intrinsic rates of automaticity. With this preparation, synchronous activation was established when \( r_J/R_{in} \) was \( \approx 10 \) (\( r_n \), 20 M\( \Omega \); \( R_{in} \), 1.5 M\( \Omega \)). This value is in good agreement with the limit for impulse propagation in adult ventricular cell pairs.

It is useful to compare the experimental cell pair data with the predictions gained from numeric simulations of impulse propagation. It should be noted, however, that the validity of these computations depends critically on assumptions about cell geometry and passive and active membrane properties (e.g., see Joyner\(^{31} \)). Several investigators adopted a segmented model consisting of two cellular domains with excitable membranes connected by a single resistance.\(^{32–34} \) Lieberman et al.\(^{35} \) using linear cables as cellular domains, reported a ratio of \( r_J/R_{in} \) of 6 as an upper limit for successful impulse transfer. Employing single cells as cellular domains, Joyner and van Capelle\(^{36} \) found that the requirement of successful transmission was met with ratios of \( r_J/R_{in} \) in the range of 1.7–10. Thus, the simulations yield critical values of \( r_J/R_{in} \) that lie between those for regular impulse transfer and complete block in cardiac cell pairs. Adopting a one-dimensional multicellular model with distributed intracellular resistance, \( r_J \), Sharp and Joyner\(^{37} \) observed intact conduction up to a 100-fold increase in \( r_J \). This compares well with the results of our experiments on cell pairs, which yielded a 150-fold increase in \( r_n \) as an upper limit.

It is conceivable that morphologically detectable connexons differ in number from functional channels. As a matter of fact, data in the literature
suggest that junctional membranes may contain connexons of different functional states. For example, experiments exploring the de novo formation of connexons between embryonic chicken heart cells revealed an open-state probability of 0.16.26 From this, it is inferred that 84% of the connexons may be silent at any given time. A similar conclusion is reached by comparing morphological and functional data applicable to cell pairs isolated from adult rat ventricles. While morphometrical considerations attribute 50,000 connexons to this preparation (assuming that disassociation and patching does not affect the number of connexons; for computation and references, see Weingart18), electrical measurements furnish 2,500–5,000 (r
, 2–4 MΩ;17, 18 single connexon conductance, 100 pS; see above). This means that 90–95% of the channels may be closed on the average. Since regular impulse transfer requires 38 connexons or more (see Table 1), a cell pair must possess at least 380–760 intercellular channels. Provided all connexons have the same kinetic properties, this implies that impulse transfer is supported by a safety factor of 65–130.

Our studies demonstrate that the functional state of impulse transfer is correlated with r
. However, it should be kept in mind that the quantitative conclusions subsequently derived must be considered cautiously. This is because distinct functional differences exist between cell pairs and multicellular preparations. On the one hand, the electric load imposed on a single cell is different in cell pairs and multicellular tissues. In the case of a cell pair, the excitatory current feeds into a large impedance element, that is, a single follower cell. In case of intact tissue, the stimulating current provides for a low impedance element, that is, a large number of electrically coupled cells. On the other hand, in multicellular preparations, at a given time there exists a large number of excited cells that can deliver current to depolarize cells at rest. Furthermore, the propagating action potential is not confined to the limits of a single cell. Its depolarizing phase extends over approximately five cell lengths.

Discrete Nature of Impulse Propagation

In a homogeneous medium, the electrical impulse propagates continuously. In a multicellular tissue, made of electrically coupled excitable cells, however, conduction is expected to be "step-wise" or discontinuous.38 Whether or not discretization is visible may depend on the contribution of r
 to the overall r (r
 = r + r ; r , cytoplasmic resistance). For example, under physiological conditions, parallel to the cellular axis, the contribution of r
 and r are comparable.18 Therefore, little or no sign of discrete propagation is anticipated. In nonphysiological situations, that is, when r
 is increased,17 discrete conduction might become apparent.39

Our studies support this concept. In cell pairs exhibiting values of r
 ranging from 5–100 MΩ, we did not observe measurable delays between action potentials. The discrete nature of impulse transfer became apparent only when r
 had increased substantially. Figure 5 illustrates an extreme case in which the transnexal conduction time was as large as 24 msec. Other investigators also reported delays in impulse propagation under conditions of elevated r
. For example, Clapham et al28 (see also Veenstra and DeHaan29), studying the synchronization of action potentials between heart cell aggregates, observed delays up to 100 msec at the onset of spontaneous activity. Their data allowed to establish a linear relation between delay and r
. This suggests that r
 must play a major role in causing delays in transnexal impulse transfer. Delays have also been observed in simulation studies (e.g., see Lieberman et al,33 Sharp and Joyner37).

In intact cardiac tissue, a transmission delay of 24 msec would lead to an extremely low conduction velocity, for example, 0.5 cm/sec in the longitudinal direction (internexal distance, 110 μm14). This is roughly 1/100 of the conduction velocity observed under normal conditions.49 This dramatic effect may be explained by the observation that cell-to-cell coupling becomes the dominating factor in controlling conduction velocity as r
 increases.35 In general, slow impulse conduction is regarded as a necessary requirement for the phenomenon of reentry, a mechanism postulated for the genesis of cardiac arrhythmias (e.g., see Cranefield40 and Janse41). Therefore, a decrease in conduction velocity via elevation of r
 may well contribute to these incidents.

Delmar et al42 explored the effects of cell-to-cell coupling on anisotropic conduction. Exposure to heptanol caused a gradual uncoupling that was associated with a stepwise decay of the conduction velocity. The authors also noticed that transverse propagation was more sensitive to an increase in r than a phenomenon that might set the stage for discrete changes in impulse conduction. Evidence supporting this view has recently been obtained by Kléber et al43 who showed that an increase in r
 is accompanied by discontinuous longitudinal propagation.

As outlined in the introduction, conduction velocity not only depends on passive cable properties, but is also determined by geometric factors and active properties of the sarcolemmal membrane (e.g., see Fozzard and Arnsdorf44). Therefore, modification of membrane excitability represents an alternative method for influencing transnexal conduction. However, so far we have made no attempts to explore this aspect with cell pairs.

Ephaptic Impulse Transmission

Based on the experimental data discussed so far, it has been concluded that transmission of an action potential from one cell to another one requires at least 13 connexons conducting simultaneously. From this it is anticipated that an electrical impulse is not propagated in the absence of a specialized junctional membrane. As a matter of fact, we were able
to verify this point directly with the following approach. Two isolated myocytes, when maneuvered into physical contact, failed to show transmission of an action potential and spread of an electrotonic response (see Figure 7). Analysis of the voltage-clamp records yielded an "intercellular" resistance of 4 GΩ. This corresponds to a conductance of 250 pS, which is roughly equivalent to two connexons in parallel (see above). This finding rules out the possibility of an ephaptic mechanism of impulse transmission in cardiac tissue. Furthermore, it does not support the concept of electric field coupling that has been propagated by Sperelakis.

Acknowledgments

We are grateful to Miss M. Herrenschwand for her expert technical assistance and to Drs. S. Weidmann, A. Kühber, and J. Shiner for critical comments on the manuscript.

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KEY WORDS • ventricular muscle • isolated cell pairs • intercellular coupling • nexal membrane resistance • action potential propagation
Action potential transfer in cell pairs isolated from adult rat and guinea pig ventricles.

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doi: 10.1161/01.RES.63.1.72

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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