IMPULSE PROPAGATION THROUGH THE CARDIAC JUNCTIONAL REGIONS OF THE AXOLOTL AND THE TURTLE

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Summary In isolated preparations from the axolotl and turtle hearts the propagation of impulses through different cardiac tissues was studied. Intracellular records were taken from the various types of fibers. Notched action potentials were recorded in two junctional regions: the atrio-ventricular ring (A–V.R.) and the ventricle-bulbus cordis (V–B.C.).

The action potential recorded in either the A–V.R. or the V–B.C. regions has a notch in its depolarization phase. The temporal occurrence of the notch was determined by the activation of the corresponding neighboring tissues. The two components given by the notch can be separated out by using different experimental variables.

The A–V.R. and the V–B.C. junctional regions are constituted by groups of horizontal fibers. When these are severed from the other adjacent cardiac tissues, it can be seen that the junctional fibers present spontaneous activity and that the action potential recorded from them is characterized by a slow rate of rise, absence of a notch, and a slow diastolic depolarization.

In these junctional regions it was found that the conduction velocity was lower than that of any of the other cardiac tissues (Table 1). These junctional regions are preferential sites for delays and blockages in propagation.

The morphological studies revealed that the cells of the A–V intermediate region are characterized by: (a) small cross sectional diameter of cytoplasmic extensions; (b) isolated and scanty myofibrils; (c) abundant glycogen deposits; and (d) sparsity of junctional contacts.

The electrophysiological and ultrastructural characteristics reported here, point towards the conclusion that in the axolotl and turtle hearts the mechanisms that underlie the delays in propagation in the junctional

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regions are similar to those of the mammalian in spite of the absence of specialized conductive tissues in the lower species.

In previous papers (Alanís and Benítez, 1964a, b, 1970; Benítez and Alanís, 1970; Martínez-Palomo et al., 1970) we have analyzed some aspects of the propagation of impulses through different cardiac tissues of the mammalian heart. Among other factors, the complex histological features of the regions in which propagation becomes critical make it difficult to carry out a detailed experimental analysis of the mechanisms involved in the propagation of impulses. On the other hand, no specialized conductive tissue similar to that of the mammalian heart is present in the hearts of reptilia and amphibia such as the turtle (Kinosternon hirtipes) and the axolotl (Ambystoma tigrinum), in which no A.V. node or Purkinje cells intercalated between the atrium and the ventricle have been reported (see Chiodi and Bartolami, 1967). This fact suggests that the cellular organization of amphibian and reptilian hearts might be less heterogeneous than that of the mammalian heart and therefore a better model for the electrophysiological study of the atrio-ventricular delay present in these species as well as in mammals. It was found however, that the mechanisms responsible for this delay are apparently as complex as those in mammalian hearts. Compound (notched) action potentials different from those generated in neighboring fibers were found in the auriculo-ventricular junctional regions in the turtle and axolotl, and similar transmembrane potentials were obtained from the junction of the ventricle with the bulbus cordis in the axolotl heart. As the main delays in propagation occurred precisely in these junctional regions, it was considered of interest to explore them further.

METHODS

The hearts were excised and fixed in a chamber with oxygenated flowing Ringer solution at room temperature (18–21°C). The composition of the Ringer solution (in mm) was as follows: NaCl, 115; KCl, 2.5; CaCl2, 1.8; NaHCO3, 2.5. The pH of the solution was adjusted to 7.2–7.3 with HCl. The hearts were opened longitudinally and spread over the bottom of the chamber with the endocardial surface upwards. For the axolotl heart a Y-shaped strand formed of the auricle, the ventricle, and the bulbus cordis was isolated (Fig. 1A) in order to analyze the sequence of activation of these tissues. To study separately the atrio-ventricular propagation in both the turtle and the axolotl, a small strand (approximately 0.5–1.0 mm in width and 10–15 mm in length) from this region was dissected under the microscope. This dissection revealed that the auriculo-ventricular ring is formed of fibers that can be classified according to their direction and gross appearance (Fig. 1B), as follows: (a) In the upper part, the auricular fibers approach the auriculo-ventricular ring at a right angle and before reaching it bend gradually to become parallel to the ring (zone 1); (b) contiguous to this auricular
tissue there is another group of parallel horizontal fibers that runs all along the preparation (zone 2); (c) the third group (zone 3) is a mirror image of zone 1, the fibers run parallel from where they are attached to those of zone 2 and thereafter they bend downward; (d) at the lower end of zone 3 the typical ventricular fibers are attached to the preceding group (zone 4). For the study of propagation from the ventricle to the bulbus cordis, a small strand (1–1.5 mm in width; 10–15 mm in length) of the other branch of the Y-shaped preparation was dissected.

The cardiac tissues were stimulated through platinum electrodes with square pulses delivered by a Grass S4A stimulator. In a few experiments no stimuli were applied, the preparations being allowed to show their spontaneous activity. Conventional microelectrodes (LING and GERARD, 1949) were used for intracellular recording (20–50 Megohms). The micropipettes were connected to one end of a P18 DC Grass preamplifier through a cathode follower (grid current 10⁻¹²A). The records were taken from a dual beam oscilloscope screen (Tektronix 502A). Specimens from the various regions of both the turtle and the axolotl hearts were processed for electron microscopy as described earlier in detail (MARTÍNEZ-PALOMO and MÉNDEZ, 1971).

RESULTS

Action potentials from different cardiac regions

When either the axolotl or the turtle hearts were driven at a constant rate through the auricle, and records were taken from the auricle, ventricle, and bulbus
cordis cells, it was observed that in general their corresponding action potentials have similar characteristics to those described for other poikilothermic species. The auricular response for example, is faster than that of the ventricle but, in turn, the latter is faster than the bulbus cordis response. On the other hand, the action potentials from the junctional regions are different in shape from those belonging to the auricle, the ventricle or the bulbus cordis. The two regions in which these special action potentials were recorded are the atrio-ventricular and the ventricle-bulbus cordis junctions. We shall subsequently designate as the atrio-ventricular ring (A–V.R.) the junctional region constituted by the lower arcuated auricular fibers (Z1 of Fig. 1B) that make contact with a strand of horizontal fibers (Z2) which in turn connects with the upper arcuated ventricular fibers of Z3. As can be seen in this figure, these groups of fibers can be distinguished by their orientation and also by their characteristic transmembrane potential (Fig. 1B). In a similar manner it was possible to differentiate, through microscopic observation, a group of fibers located between the ventricle and the bulbus cordis which run perpendicular to the neighboring ventricular fibers. These horizontal fibers formed together with the adjacent segments of the ventricle and the bulbus cordis what will be designated from now on as the ventricle-bulbus cordis junctional region (V–B.C.).

The action potentials recorded from either the A–V.R. or the V–B.C. junctional

Fig. 2. Action potentials from different cardiac regions. Axolotl at the left; turtle at the right. Note that the potentials from the A–V. R. fibers (2, 3, 4 in axolotl; 2, 3 in turtle) have either a slow rate of rise or a notch. The special shape of these action potentials permits to differentiate them from the typical responses of the auricle and ventricle (1, 5 in axolotl and 1, 4 in turtle). Calibration: 200 msec, 50 mV.
Fig. 3. Transmembrane potentials from the ventricle-bulbus cordis junctional regions of the axolotl. Observe that the intermediate action potentials (2, 3, 4) have distinguishing features as compared with the ventricle (1) and the bulbus cordis (5) responses. Calibration: 200 msec, 50 mV.

Fig. 4. Separation of the two components of an action potential from the A–V. R. junctional region in the axolotl heart. Upper trace, action potential from an intermediate fiber adjacent to the auricle. Lower trace, auricular response. In A, the auricle was activated at a low frequency. Observe that the action potential of the intermediate fiber has a fast component simultaneous with the upstroke of the auricle and a slow component that corresponds to the intermediate fibers activity. When the auricle was stimulated at a higher frequency (in B), it was observed that the notch of the intermediate potential was first delayed and afterwards disappeared when the intermediate fibers failed to follow the high frequency. Calibration: 100 msec, 20 mV.

regions have as a distinguishing characteristic a notch in their depolarization phase. These action potentials are formed of two components with different time courses (Figs. 2, 3). The temporal occurrence of the notch was closely related with the activation of either the auricle or the ventricle when the records were taken from the upper or lower part of the A–V.R. In the case of the V–B.C.
Fig. 5. Experimental conditions as in Fig. 4. Records taken from the auricle (lower record) and from the intermediate fibers (upper record) of the turtle heart. Note in A, at the arrow, the notched upstroke of the action potential from the intermediate region. As the frequency of stimulation was increased, the second component appeared later (B, C) and finally alternated, since the intermediate fibers failed to respond to each stimulus. Calibration: 250 msec, 50 mV.

junctional region, the notch appeared only when either the ventricle or the bulbus cordis were active. The two components of the action potential just described may be separated out by different experimental procedures: progressive increasing of the frequency (Figs. 4, 5), addition of acetylcholine (0.5 µg/ml) to the chamber (Fig. 6), perfusion with hyperosmotic (saccharose 100–250 mM) or with calcium-free Ringer solutions, or severing of the junctional regions from the corresponding neighboring tissues leaving only the Z2 horizontal fibers of the A–V.R. or the intermediate segment of the V–B.C. junctional regions.

The isolation of either group of horizontal fibers revealed that they present spontaneous activity. The action potential recorded from these fibers was characterized by a slow rate of rise, absence of the notch, and a marked slow diastolic depolarization. The frequency of pacemaker activity originated in the horizontal fibers of the A–V.R. and the V–B.C. regions was in general lower than that of the normal pacemaker of the intact heart. As in the mammalian heart there is a gradient in the frequency of automatic activity, since the intermediate region of the V–B.C. junction has the lowest frequency of spontaneous beating.
Fig. 6. Acetylcholine and propagation of impulses through the ventricle-bulbus cordis junction. Stimulation of the ventricle at a constant frequency (0.7/sec). In A, action potential from a fiber of the intermediate region. Note the slow rate of rise and the slight notch at the end of the depolarization phase. In B, same potential during perfusion with acetylcholine (0.5 µg/ml). Note that the notch is more evident and appears later due to the slowing in propagation. Calibration: 100 msec, 20 mV.

Sequence of activation of the different cardiac tissues

When the auricles of both the turtle and axolotl hearts were driven at a constant frequency, and simultaneous recordings with two micropipettes were taken from the auricle and ventricle it was seen that the impulses conduct at different rates through the cardiac tissues. In the Y-shaped preparation of the axolotl there are two regions in which propagation is extremely slow, i.e., the A-V.R. and the V-B.C. (Fig. 7). In the turtle heart the longest delay appeared in the A-V.R. The conduction velocity measurements of each of the tissues involved in propagation confirmed that in both the axolotl and the turtle hearts, the lowest speed was recorded at the above-mentioned junctional regions (Table 1). As can be observed in the table, the conduction velocity of either the auricle or the ventricle is several times greater than that of the A-V.R. Similarly, at the junction between the ventricle and the bulbus cordis the conduction velocity was one-seventh and one-fourth of that of the ventricular muscle and bulbus cordis respectively. These junctional regions behave as preferential blocking sites for propagation. Direct stimulation of either the auricle or the ventricle with progressively increasing frequencies revealed that each of these tissues responded to

<table>
<thead>
<tr>
<th>Specie</th>
<th>Atrium</th>
<th>Atrio-ventricular junctional fibers</th>
<th>Ventricle</th>
<th>Ventricle-bulbus cordis junctional fibers</th>
<th>Bulbus cordis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axolotl</td>
<td>0.115 (14)</td>
<td>0.004 (12)</td>
<td>0.037 (14)</td>
<td>0.0002 (8)</td>
<td>0.009 (8)</td>
</tr>
<tr>
<td>Turtle</td>
<td>0.158 (20)</td>
<td>0.028 (7)</td>
<td>0.085 (7)</td>
<td></td>
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Average values from a number (figures in parentheses) of experiments.
frequencies up to 6 and 4/sec, respectively, but when the ventricle was activated by impulses coming down from the auricle it could only follow frequencies up to 1.5/sec; while the horizontal fibers of Z2 (Fig. 1B), which are located between the auricle and ventricle, could respond to a higher frequency (2.3/sec). The other critical region for propagation is the V–B.C., which behaves similarly to the A–V.R. when subjected to the same experimental conditions; that is, when increasing frequencies were applied to the ventricle and the corresponding responses of the ventricle and bulbus cordis were simultaneously recorded, it was observed that initially the latency between their responses was gradually lengthened (Fig. 8) until the bulbus cordis failed to respond. This occurred at a frequency lower than that which the bulbus cordis could follow when it was directly stimulated. For example, in the experiment illustrated in Fig. 8, the bulbus cordis when activated through the ventricle failed to respond to a frequency of 1.7/sec, but when it was directly stimulated it responded up to 2.3/sec. Other variables such as acetyl-
Fig. 8. Changes in the interval between ventricle and bulbus-cordis responses when increasing frequencies were applied to the ventricle. Typical action potentials of ventricle and bulbus cordis (inset, calibration: 145 msec, 40 mV) in response to the activation of the ventricle at low frequency (1/sec). As can be seen in the curve, the latency lengthened gradually as the frequency increased. The bulbus cordis failed to respond (arrow B) while the ventricle failed at a higher frequency (arrow A). Ordinates, magnitude of V–B.C. latency in msec; abscissae, reciprocal of the frequency of stimulation, in msec.

Morphological findings

Atrial and ventricular cells of the axolotl and the turtle hearts have a mean cross sectional diameter of 5 μ at the cell body, where the nucleus is located (Fig. 9). Most of the cytoplasm is occupied by regularly disposed myofibrils. The intervening spaces between myofibrils show numerous mitochondria and abundant deposits of glycogen granules. A clear difference between atrial and ventricular cells is found only with respect to the relative number of specific granules, which are found to be more abundant in atrial cells. Numerous desmosomes and intermediate junctions are found along regions of side-to-side and end-to-end contact between adjacent atrial or ventricular cells (Fig. 11). In amphibian and reptile heart muscle cells, the specialized intercellular junctions do not form a continuous row of end-to-end cell junctions similar to the intercalated disc of mammalian cardiac cells. Gap junctions can be observed in both atrial and ventricular cardiac cells, as reported earlier (Martínez-Palomo and Méndez, 1971).

Cardiac cells of the A–V junctional regions were identified in serial sections in which the hole produced by the micropipette tip that recorded a notched action
Fig. 9. Low magnification electron micrograph showing four turtle ventricular muscle cells (C_1 to C_4). The cells are characterized by their narrow diameter and the presence of a prominent nucleus (N). The cytoplasm is mostly occupied by regular bundles of myofibrils. The paranuclear zone presents numerous rod-shaped mitochondria (Mi) and varying amounts of glycogen deposits. Lateral intercellular spaces (arrows) present numerous regions of membrane apposition between adjacent ventricular cells. Bar equals 1 μ. 
Fig. 10. Cardiac cells from the axolotl A–V junctional region. Under the electron microscope the cytoplasm of the A–V cells is found to be characterized by the sparsity of myofibrils (My), the presence of large mitochondria with circular profiles (Mi), abundant glycogen deposits and various membrane-limited intracytoplasmic vacuoles (V). There is a large and oval nucleus (N) and few junctions between adjacent cells (arrowheads). Bar equals 1 μ.
Fig. 11. High magnification electron micrograph of a region of intercellular contact between two axolotl ventricular cells. Desmosomes (D) and intermediate junctions (IJ) are irregularly distributed between adjacent cell membranes. Bar equals 0.1 μm.

Fig. 12. High magnification electron micrograph of a region of close approximation between two axolotl A-V cells. In contrast with the abundant cell junctions found between ventricular or atrial cardiac cells, junctional elements are usually absent at the sites of cell-to-cell apposition between converging cell membranes at the A-V junctional region. Bar equals 0.1 μm.
potential had been found. Under the electron microscope, the cells of the A–V region (Fig. 10) were found to possess distinct morphological features. The cross sectional diameter at the cell body was similar to that of atrial and ventricular cells; however, the cytoplasmic extensions were in general narrower and followed a more tortuous course. The cytoplasm shows very few isolated myofibrils with no particular arrangement with respect to the cell form. Mitochondria are few, small, and round; most of the cytoplasm is occupied by varying amounts of glycogen particles. An additional distinctive feature of particular interest was the striking sparsity of cell junctions between these cells (Fig. 12). Both desmosomes and intermediate junctions are only infrequently seen between adjacent A–V cells and no gap junctions were identified with certainty.

**DISCUSSION**

The present results give additional support to the interpretations advanced by several authors (Hoffman and Cranefield, 1960; Sano et al., 1964; Matsuda et al., 1967; Alanis and Benítez, 1967, 1970; Martínez-Palomo et al., 1970) in regard of the mechanisms responsible for the delays that appear in certain regions of the mammalian heart and in the cold blooded species (Shinozaki, 1942; Inoue, 1959; Kanno, 1963; Tsuji, 1963; Irisawa et al., 1965). We have shown that in the axolotl and turtle hearts there are limited regions in which the propagation of impulses is delayed. The regions in which these delays occurred are the junctional regions (A–V.R. and V–B.C.) in which, moreover, action potentials with a slow rate of rise and a reduced amplitude were recorded. Similarly, it was demonstrated that several variables may decrease the conduction velocity or block the propagation in these critical junctional regions. These findings agree with the interpretation that the characteristics of the transmembrane potentials generated in the junctional cardiac fibers contribute to the determination of the rate of conduction. At the same time it was considered important to study the histology and fine structure, by means of light and electron microscopy, of the elements through which the impulses propagate, in order to determine whether the cells of these critical intermediate fibers differ morphologically from other cardiac cells involved in propagation. As was described above, the intermediate cells of the A–V.R. junctional region differ in fine structural features from adjacent fibers, particularly as to diameter, the quantity of myofibrils and the distribution and number of specialized intercellular junctions. Moreover, in an earlier paper (Martínez-Palomo et al., 1970) it was reported that in the dog heart there are cells located between the Purkinje and ventricular fibers which generate transitional potentials markedly different in configuration from those originated in the Purkinje or ventricular cells. These junctional cells have some distinguishing cytological characteristics that, according to the electrical theory, may determine the slow rate of conduction. The three most important of these characteristics are : (a) the absence
of a transverse tubular system; (b) the small diameter of the cell; and (c) the sparsity of specialized intercellular junctions.

It will be seen that there is a striking similarity between the mammalian and the lower vertebrate species as to the electrophysiological and ultrastructural characteristics of the junctional cardiac regions. In view of these special characteristics, it may be concluded that the safety margin for the propagation of impulses across the intermediate cells might be low and the excitation of the corresponding adjoining tissues would consequently be easily impaired. This is further indicated by the fact that variables of different types, such as increasing the frequency of stimulation, addition of acetylcholine to the chamber, and the use of calcium-free or hyperosmotic perfusing solutions, all affected conduction deleteriously.

The action potentials from the intermediate fibers of the A–V.R. and V–B.C. regions are influenced to a certain extent by the activation of neighboring tissues. The contribution of these adjacent active tissues was evidenced by the presence of a notch in the action potential of the intermediate fibers and by the simultaneity of the notch with the upstroke of the response of one of the neighboring tissues. In the experiments in which the intermediate fibers were separated out from the adjoining tissues by means of a surgical procedure, it was shown that each cell generates a typical potential different from that recorded before the separation. The transmembrane action potential of these intermediate fibers does not present notches and its depolarization phase has a slow rate of rise. The slow rate of rise of these potentials might explain by itself the slow conduction velocity through the intermediate regions and would suggest in addition that their transmembrane potentials are probably generated by a slow ionic channel mechanism. This fact would be in contrast with the potentials of the other cardiac tissues in which a fast sodium channel mechanism is known to be involved during the upstroke.

We do not know, however, whether under normal conditions in the in situ heart the electrotonic interaction between the adjacent tissues and the intermediate fibers plays a role in the propagation of impulses. This question could only be answered if the space and time constants of the junctional cardiac regions were measured.

We would like to make a comment in regard to the notched action potentials recorded at the junctional regions. It is a well-known fact that two or more components may appear in an action potential recorded even from homogeneous nerve fibers (HODGKIN, 1937) which have been blocked by low temperature or by a narcotic. In our own experience (unpublished observations) a notch can be obtained in the action potential of any of the cardiac tissues whenever the conduction velocity is made extremely slow by experimental procedures such as the use of cold, anoxia, etc. However, if one is able to show that the notch is in close temporal relation with the activation of a different tissue located very near the cell from which the records are taken, then the notch might have a functional meaning, since it would give an idea of the magnitude and the temporal occurrence of the
electrotonic spread in a heterogeneous conductor such as the cardiac tissues. For instance, potentials with notches can be obtained under normal conditions in all the junctional cardiac regions of the mammalian and lower species, as well as in other heterogeneous structures, such as the neuromuscular junctional region (Eccles et al., 1941; Fatt and Katz, 1951), septal commissural junctions of lateral giant axons in the crayfish (Watanabe and Grundfest, 1961), and cat motoneurones (Coombs et al., 1957).

As a working hypothesis, we had assumed that the complexity of the mechanisms of propagation in mammalian cardiac tissues is partially due to the great variety of cellular groups with different structural characteristics. This hypothesis is not borne out by the present study. Though the specialized conductive tissues of mammalian hearts are not present in these lower species as a distinct anatomical entity, the complexity in the mechanisms of propagation still remains. In spite of the differences in morphology between the poikilotherm and mammalian hearts, we are inclined to conclude that the electrical coupling of the cardiac cells and the propagation of impulses through them are similar in the two.

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REFERENCES


