The Spread of Excitation in the Embryonic Chick Heart

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ABSTRACT The spread of excitation in embryonic chick hearts, ranging in age from 7 to 20 days, was studied with both intracellular and extracellular electrodes. Evidence that the delay in ventricular excitation could be attributed to the cells of the entire atrioventricular (AV) ring was obtained, in part, from sagittal sections of the heart. In the intact preparation, uniform propagation occurred throughout the atrial roof at an apparent conduction velocity of 0.4 to 0.5 meter/sec. Delay of impulse propagation was localized in a very narrow band of tissue which extended across the AV ring. The apparent conduction velocity of this tissue was between 0.003 and 0.005 meter/sec. Both normal and retrograde propagation revealed the spread of conduction across the AV ring to be decremental in nature. This finding was supported by high frequency stimulation experiments which gave rise to AV block localized in the cells of the AV ring. Cardiac rhythmicity and AV transmission were responsive to acetylcholine and norepinephrine in much the same manner as in the adult mammalian heart. The present findings are in support of the hypothesis that the embryonic AV ring is the functional counterpart of the adult AV node.

INTRODUCTION

Studies concerned with the sequence of activation and nature of propagation delay in the embryonic chick heart have thus far been limited to visual and electrocardiographic observations. In 1890, Fano and Badano (4) reported the occurrence of transmission from atrium to ventricle when the embryonic heart was cut in zigzag strips, and AV continuity maintained. This was proof that excitation could spread to the ventricle from any point along the embryonic AV junction. Pickering (19) noted the sequential contraction of different parts of the embryonic heart and questioned the presence of a slowly conducting tissue across the AV junction. In 1913, the first recording of electrical activity was obtained from embryonic hearts (23). The electrocardiogram of 3 and 4 day embryonic chicks subsequently revealed the pres-

ence of a PR interval in the absence of the AV node (6, 21). In 1956, Patten (18) summarized the major contributions regarding the origin and propagation of the embryonic heart beat. Since that time, electrocardiograms from 72 hour chick embryos have confirmed the presence of AV delay (1, 17).

The technique of intracellular recording has enabled investigators to study, in detail, the origin and spread of excitation in the adult mammalian heart (15) and the transmission of excitation through the AV node (8, 9, 14). Although intracellular recordings have been obtained from embryonic chick hearts at various stages of development (5, 10, 12), neither mapping of the excitation spread nor the nature and mechanism underlying the atrioventricular delay have been investigated. An understanding of the manner in which the heart beat is propagated through the seemingly "unspecialized" embryonic heart will undoubtedly be useful in furthering our comprehension of the developmental processes which give rise to the formation of the adult heart. In the present study, modern electrophysiological techniques are employed in mapping the excitation spread in embryonic chick hearts of various ages.

METHODS

The same preparation and apparatus described previously (10) have been used in the present investigation. The spread of excitation was studied in preparations driven by stimuli applied through a pair of electrodes situated in the atrial roof, at frequencies between 2.5 to 3.5 cycles/second. The driving stimulus was kept at a constant rate during each experiment. Bipolar electrograms from the endocardial surface of the right atrium and right ventricle were recorded by two fine teflon-coated silver electrodes connected to a DC amplifier with an adequate frequency response (0 to 1 kc). The exploring electrode was moved in a straight line along the pectinate muscle of the atrial roof and its exact position was noted (to the nearest tenth of a millimeter) for every impalement. The electrode was subsequently advanced in a straight line perpendicularly across the AV ring and AV valve. The activation time to each recording site was considered as the time between the stimulus artifact and a point at 50 per cent of the total upstroke amplitude. From this information, graphs of the spread of activation across the atrium, AV ring, and AV valve were constructed. This technique was adapted from the isolated rabbit heart studies of Paes de Carvalho *et al.* (14, 15).

Acetylcholine (acetylcholine chloride) and norepinephrine (levarterenol bitartrate) were added separately to the perfusate in amounts which brought the final concentration to 20 μg/ml (Ach) and 8 μg/ml (N.E.). 0.1 mM EDTA (disodium salt) was added to the norepinephrine-containing solution to delay its oxidation.

RESULTS

A. *The Spread of Excitation*

Fig. 1 relates tracings of transmembrane potentials from several areas of a 16 day heart to an electrogram recorded from the endocardial surface of the

atrium and ventricle. Representation of data in this fashion shows the sequence and timing of single fiber activation in the embryonic heart.

Mapping the spread of electrical activity in the right atria of 13 to 19 day embryonic hearts demonstrates uniform propagation from the point of stimulation at an apparent conduction velocity of 0.4 to 0.5 meter/sec. (Fig. 2). The time of application of the driving stimulus to the atrium is considered the zero time.

For determining the spread of activity across the AV ring and valve, the

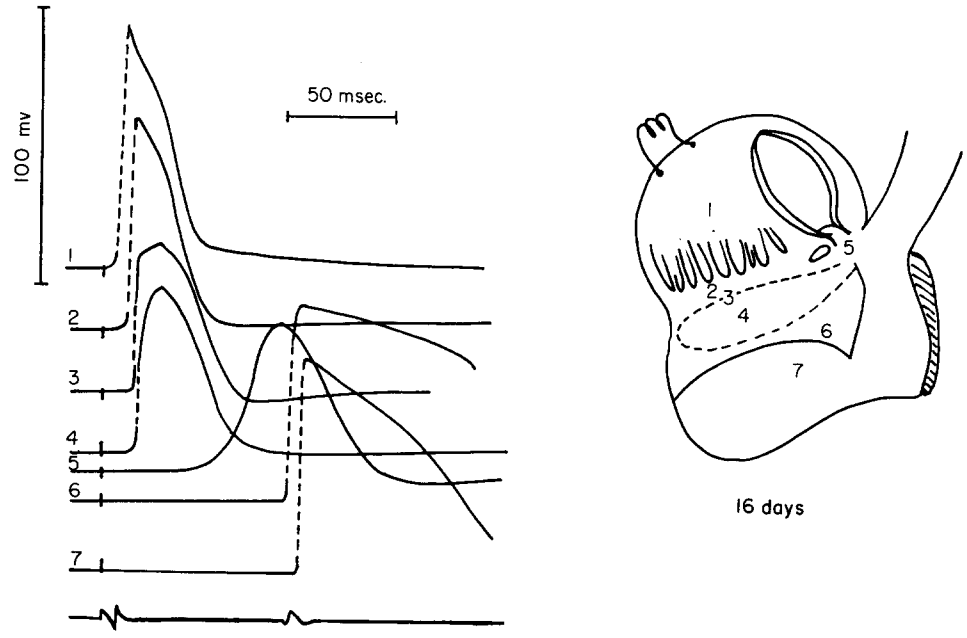

FIGURE 1. Tracings of transmembrane action potentials and bipolar electrogram recorded from a 16 day chick embryo heart.

instant of cell activation at the atrial border of the AV ring is regarded as the zero reference for the timing of propagation. It is possible to record from cells of the AV ring and, with deeper penetration, from the ventricular portion of the AV valve. This procedure results in two widely separated activation times for cells at the same locus (Fig. 3). The left side of the graph represents the gradual increase of activation time as the wavefront of activity progresses across the upper portion of the AV ring. Almost all of the AV delay is localized in a narrow band of tissue (N region (10)) which extends for a distance of approximately 0.2 mm along the entire AV ring. The conduction velocity across this strip of tissue is between 0.003 and 0.005 meter/sec. and propagation through this region accounts for approximately 50 msec. of the total AV delay. Excitation leaving this region proceeds to the NH zone and then, rather quickly, to the AV valve cells beneath the AV ring. It is from

the latter cells that the data to the right of Fig. 3 are obtained. The significance of the jump in activation time becomes apparent if consideration is given to the hypothesis that the cells of the ventricular portion of the AV valve are precursors of the His-Purkinje system (10). Although not extensively studied, the conduction velocity in the right ventricle is of an order of magnitude similar to that found in the right atrium; *i.e.*, 0.4 to 0.5 meter/sec.

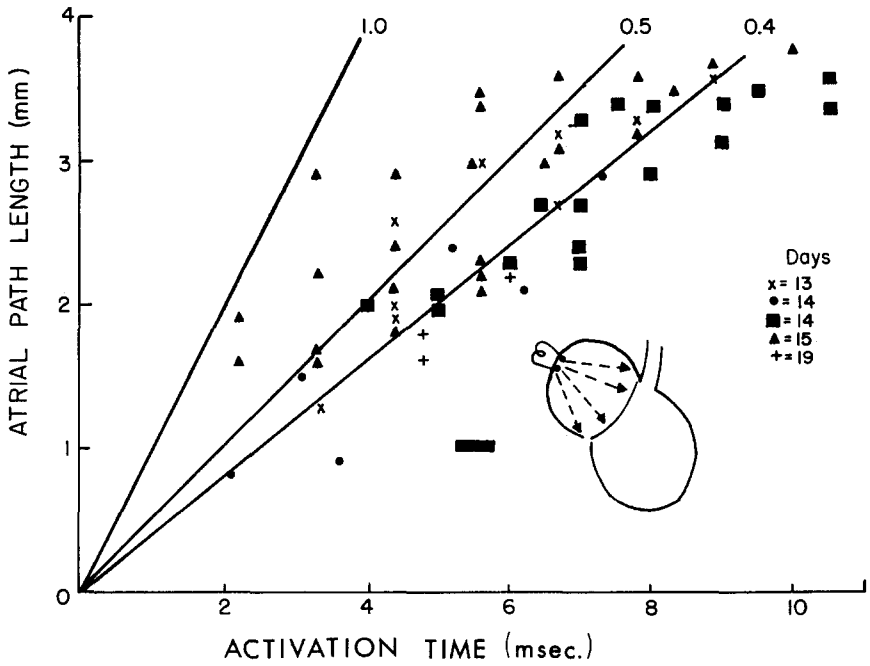

FIGURE 2. Spread of activity across the right atrium. Horizontal bar represents limits of accuracy in measurements (0.5 msec., 0.1 mm). Solid lines are the geometric loci of data points corresponding to propagation velocities of 0.4, 0.5, 1.0 meter/sec. Insert shows approximate direction of propagation.

A graph similar to the one already described was obtained from preparations of 7 and 8 day hearts. The delay of impulse propagation could be localized in a very narrow band of tissue along the AV ring with an apparent conduction velocity of approximately 0.003 to 0.005 meter/sec. It was not possible to map the spread of excitation in hearts less than 7 days of age because of the limitations imposed by the dimensions of the heart.

In order to determine whether or not the delay in ventricular excitation is a characteristic function of the entire embryonic AV ring musculature, sagittal strips of late embryonic heart muscle were prepared by an incision across the atrium, AV valve, and ventricle. Muscular continuity was maintained between atrial and ventricular portions. The strips were electrically

driven and both intra- and extracellular recordings were obtained by previously described methods. This study reveals PR intervals even from strips of the most lateral segments of the preparation in addition to typical AV ring cells whose sequence of activation is similar to the intact preparation.

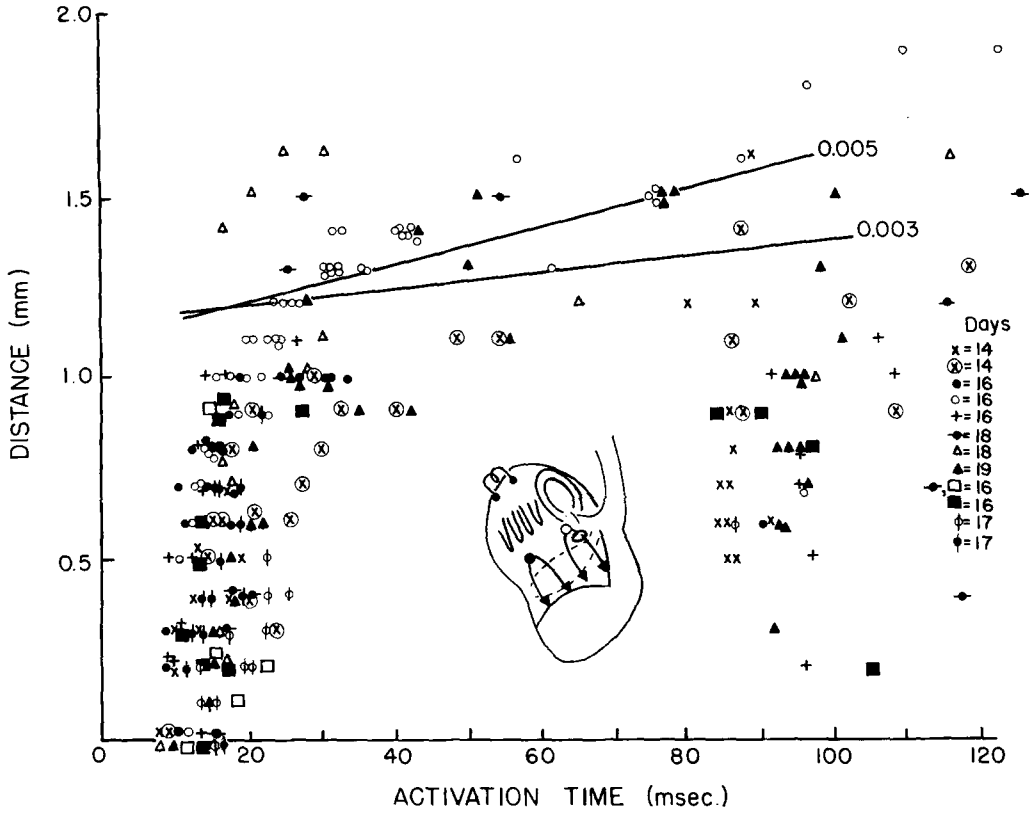

FIGURE 3. Activation of the embryonic AV ring and AV valve. Solid lines are the geometric loci of data points corresponding to propagation velocities of 0.003 and 0.005 meter/sec. Insert shows lines along which records were taken. Zero is the time of activation of the atrial margin of the AV ring.

B. *The Mechanism of AV Transmission*

The mapping data presented above suggest that the impulse is considerably delayed as it traverses the middle region of the AV ring. In order to reveal the mechanism of propagation within the embryonic AV ring, two types of experiments were performed.

In one experiment, the preparation was alternately driven at the same frequency from the atrium (forward) and ventricle (retrograde) while transmembrane action potentials were recorded from cells of the atrium and AV

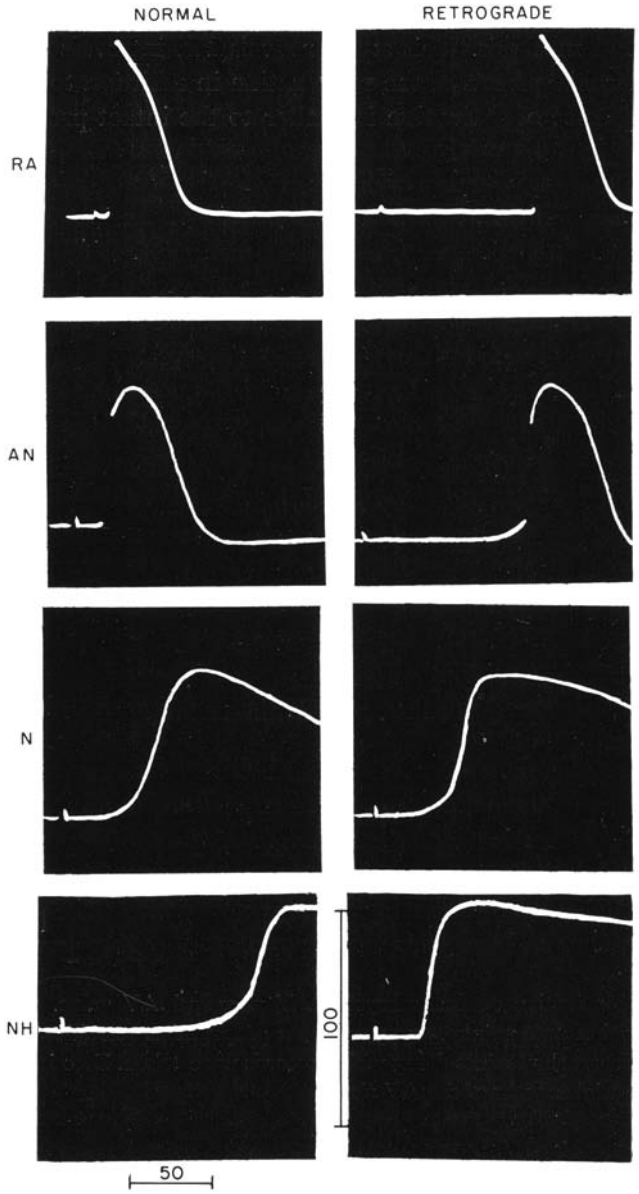

FIGURE 4. Transmembrane action potentials recorded from the AV ring during normal and retrograde propagation. Horizontal bar = 50 msec. Vertical bar = 100 mv.

ring. Records obtained from the same cell during normal (forward) and retrograde drive are shown in Fig. 4. The shape of action potentials recorded from right atrial cells is independent of the direction of propagation. However, each of the AV ring cells, depending on the direction of excitation, responds differently. In the cells of the AN region, the transition from the

diastolic period (phase 4) to the upstroke (phase 0) is slower when driven from the ventricle (presence of a slowly rising "foot") than when driven from the atrium. The maximum rising velocity appears to be of the same magnitude in both directions of excitation. The cells in the N region exhibit a slow foot during normal and retrograde drive. In the record shown, a higher rising velocity is also observed in the retrograde potential. In the NH region, a slow foot is evident only during forward excitation. These records are similar to those obtained from comparable areas of the adult rabbit heart (14).

In the second type of experiment, the preparation was stimulated at progressively higher frequencies during which time intracellular and extracellular potentials were recorded simultaneously. An increase in the rate of stimulation is known to depress AV nodal conduction by "fatigue" of the N cells in the rabbit heart (13). Transmembrane potentials obtained simultaneously from an atrial cell (upper trace) and a ventricular cell (lower trace) are displayed in Fig. 5. As the frequency of stimulation gradually increases (Fig. 5A to C), the time between atrial and ventricular excitation is prolonged and 2:1 AV block finally develops.

It is possible to localize the transmission failure by simultaneously recording action potentials and surface electrograms during progressive increases in the rate of stimulation. The upper trace in Fig. 6 shows the response to a progressive rate increase from cells of the right atrium (A), atrial margin of AV ring (B), and middle AV ring (C), while the lower trace displays a bipolar electrogram complex. Right atrial cells are capable of producing a propagated response when stimulated at high rates. The action potentials decrease in amplitude at the same time the conduction velocity apparently diminishes in the atrial tissue. Potentials from the upper AV ring area are not greatly altered in shape when the rate of stimulation produces an increase in AV transmission time and AV block. However, at very rapid driving rates, cells from the upper ring area exhibit an irregular electrical response that might be caused by either (*a*) the change in electrical activity which occurs in the atrial cells, (*b*) the dependence of the ring cell potentials on the duration of the preceding phase 4, or both (*a*) and (*b*). Records from a fiber in the middle AV ring show a steplike depolarization which occurs simultaneously with an increase in AV transmission time. The subsequent action potential is markedly diminished in amplitude, duration, and rising velocity when the electrogram reveals a failure of propagation. A further increase in the rate of stimulation produces a slowly rising, low amplitude, steplike potential change. This response does not give rise to a local action potential and occurs simultaneously with complete failure of AV transmission.

Electrical activity also was recorded from cells of the ventricular portion of the AV valve and right ventricle during increasing rates of stimulation. The results obtained not only substantiate the above mentioned data, but also

verify a well known event which occurs in the adult heart when the ventricle is stimulated directly at a rapid frequency. Retrograde stimulation produces complete heart block at a rate which results in a 2:1 block during normal propagation. This indicates that impulses propagate across the embryonic AV ring with less difficulty in a forward rather than a retrograde direction.

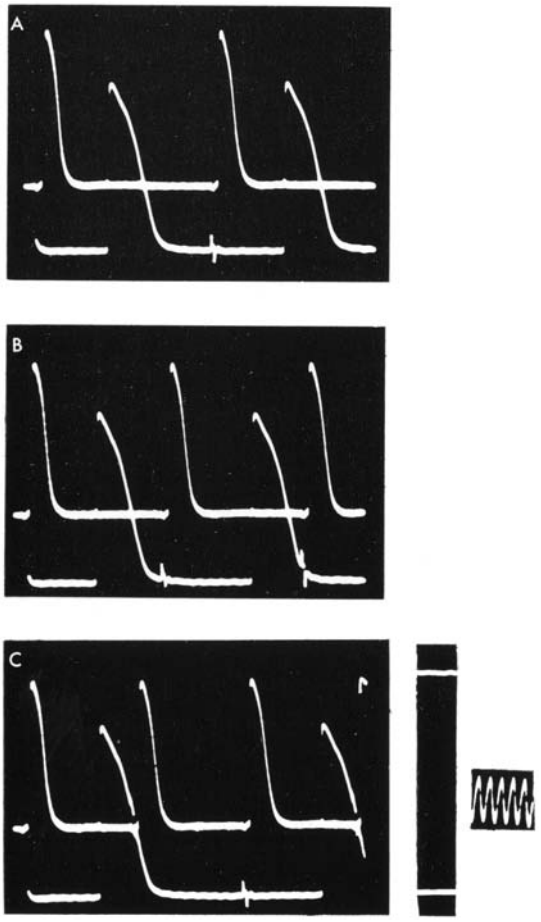

FIGURE 5. Transmembrane action potentials recorded simultaneously from right atrium (upper) and right ventricle (lower) during progressive increases in the rate of stimulation (A to C). Voltage calibration = 100 mv. Time calibration = 60 CPS.

C. *The Effects of Acetylcholine and Norepinephrine on AV Transmission and Heart Rate*

Electrograms were obtained from spontaneously beating hearts (10 to 13 days) which were bathed in normal Tyrode solution as well as in Tyrode solutions containing acetylcholine and norepinephrine. The results obtained show that acetylcholine produces a decrease in heart rate (from 184.4 ± 9.4 to 49.0 ± 19.6 beats/min.; $n = 11$ and $P < 0.001$)[1] with no apparent effect on the PR

[1] (Mean ±SE; n = No. of observations; P-value from Student "t" table).

interval (Fig. 7A). Also, cardiac arrest occurs in 70 per cent of the trials. Since a slow heart rate is known to favor AV transmission, and increasing the rate of stimulation has previously been shown to increase AV transmission

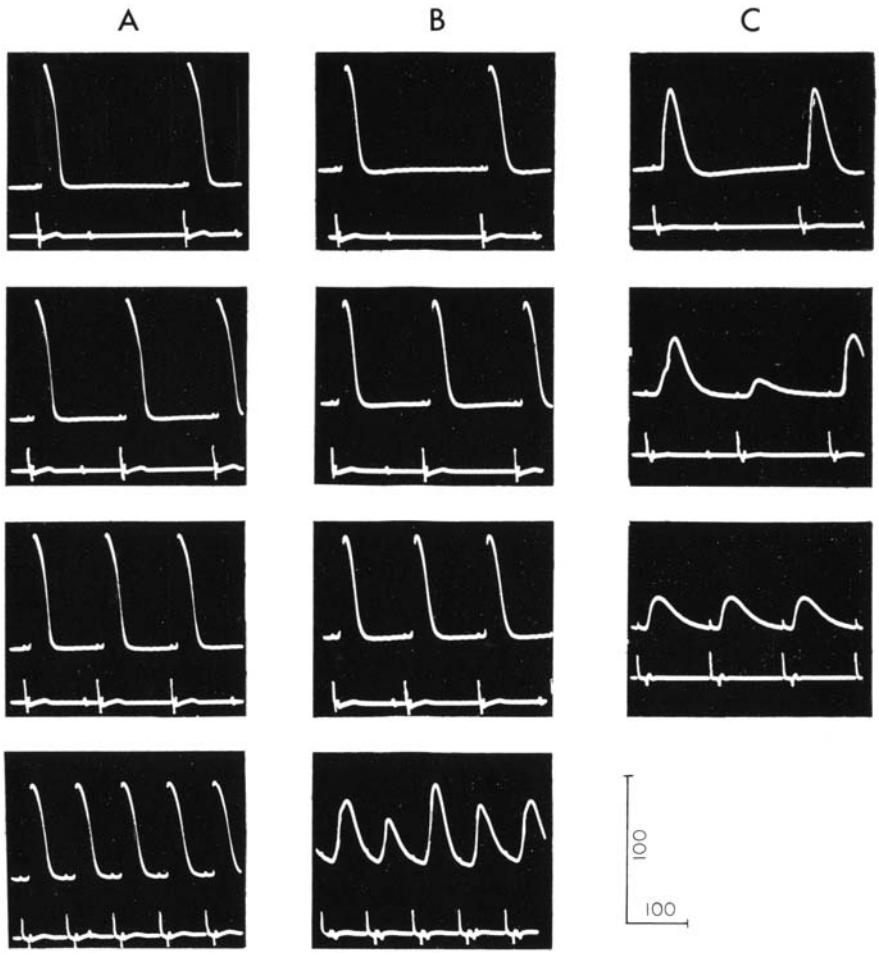

FIGURE 6. Transmembrane action potentials (upper) and bipolar (atrial and ventricular surfaces) electrograms (lower) recorded from embryonic chick heart during progressive increases in the rate of stimulation. (A) Right atrial cell, (B) atrial margin of AV ring, (C) middle AV ring. Vertical bar = 100 mv. Horizontal bar = 100 msec.

time (Figs. 5 and 6), it was necessary to drive the hearts at a fixed frequency in normal Tyrode solution and then in the presence of acetylcholine. This procedure produced a Wenckebach rhythm in some cases (Fig. 7C), and a prolonged PR interval in others. Since the transmission time is increased at a much lower frequency of stimulation than in the earlier reported studies, acetylcholine must have enhanced the "sensitivity" of the AV transmission system to the frequency of stimulation.

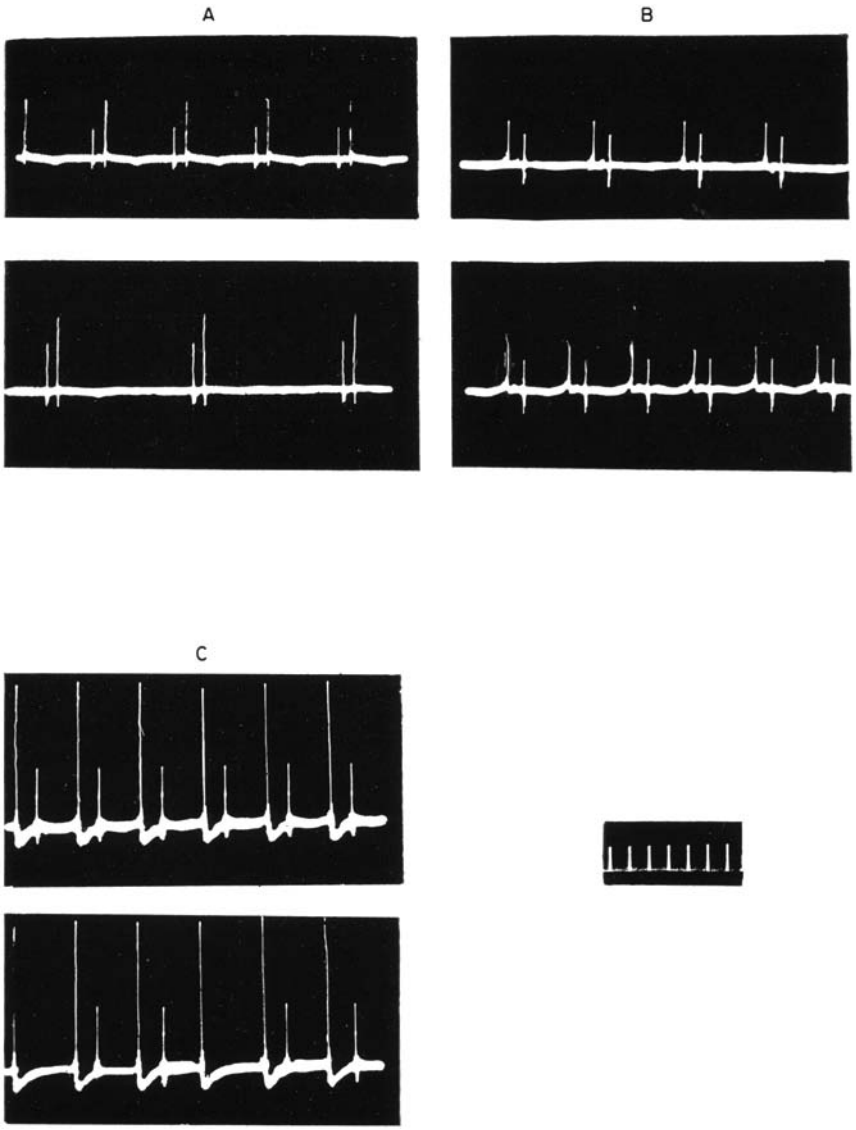

FIGURE 7. Bipolar electrograms recorded from spontaneously beating (A, B) and driven (C) embryonic hearts under the influence of acetylcholine (A and C, lower records) and norepinephrine (B, lower record). Upper trace = control. Time calibration = 100 msec./div.

The addition of Tyrode solution containing norepinephrine causes tachycardia in the spontaneously beating preparation (from 163.0 ± 15.7 to 211.3 ± 15.4 beats/min.; $n = 7$ and $P < 0.005$) (Fig. 7B). The PR interval decreases significantly in response to norepinephrine (from 74.8 ± 3.6 to 60.9 ± 3.5 msec.; $n = 8$ and $P < 0.001$), whereas the rapidly driven hearts

previously exhibited an increased PR interval (Figs. 5 and 6) in the absence of norepinephrine.

The results of the above studies with 10 to 13 day embryonic hearts show that both cardiac rhythmicity and AV transmission are susceptible to the presence of the transmitter agents, acetylcholine and norepinephrine.

DISCUSSION

A. *The Spread of Excitation*

The spread of activity in electrically driven embryonic hearts rapidly advances through the right atrium to the atrial boundary of the AV ring. It appears that the activity propagates radially and uniformly throughout the roof of the right atrium (*i.e.*, along the pectinate muscles). The results are remarkably similar to those reported for the adult mammalian heart as determined by both intracellular (15) and surface (7) electrodes. Thus, embryonic atrial muscle demonstrates an electrophysiological behavior very similar to that of the adult mammalian atria and thereby exhibits an apparent degree of functional "maturity."

As the electrical activity proceeds to activate the AV ring, propagation becomes noticeably slower, as evidenced by the increasing delay in activation (Figs. 3 and 4). The membrane potentials recorded from the AV ring permit a description of this embryonic structure in terms employed to describe the adult rabbit AV node (13, 14); *i.e.*, a functional region composed of three zones. The cells of the middle zone (N region) are characterized by action potentials which propagate with a progressive fall in rising velocity (10, 13, 14). The N region is thereby considered the slowest propagating area of the adult rabbit AV node (14) and the embryonic chick AV ring. However, the conduction velocity of the AV node (9, 22) is approximately ten times greater than that of the embryonic AV ring. This difference may be attributed at least in part to the higher temperature (35–37°C) at which the mammalian experiments were conducted.

Positive identification of the embryonic AV ring has been based on (*a*) its anatomical position with respect to the fibers of the right atrium and AV valve, (*b*) the presence of distinctive action potential configurations which differ from those recorded from the atrium and the ventricular portion of the AV valve (10), (*c*) a marked delay in impulse transmission through this region, and (*d*) the localization of AV block to specific areas of the ring. Furthermore, the graphs of excitation spread (Figs. 2 and 3) and the simultaneous records of intracellular potentials and bipolar electrograms (Fig. 6), reveal a continuous sequence of excitation from the right atrial roof to the AV valve through the fibers of the AV ring. Lastly, the embryonic heart muscle strip experiments support the earlier findings (4, 18) that AV transmission can occur along the entire AV junctional tissue.

The presence of a specialized sinoventricular connecting pathway has been debated in the literature since 1920 (20). In the course of the present investigation, membrane potentials similar in configuration to those of the AV ring were recorded from the sinus septum (Fig. 1). Although this septum was not mapped extensively, its anatomical relationship to the upper portion of the interventricular septum appeared to support the possibility of a sinoventricular conduction pathway in the embryonic chick heart. However, cutting this septum did not noticeably affect AV transmission since the AV connections throughout the ring were still intact.

B. *Mechanism of AV Transmission*

The mapping data suggest that the wavefront of excitation traverses the AV ring with considerable difficulty. However, these experiments do not define the mechanism of propagation within the AV ring. On the basis of results obtained from studies of the adult rabbit AV node, Hoffman *et al.* (9) considered conduction through this area to be decremental. This term is defined by the authors as a type of conduction in which ". . . the action potential appears to diminish progressively in amplitude and rate of depolarization in such a way as to suggest that its efficacy as a stimulus to adjacent regions is continuously reduced."

The present study includes several types of experimental procedures to reveal the nature of AV delay in the embryonic heart. When the preparation is successively driven from the atrial roof and then from the ventricle, a slow foot appears in action potentials which are activated by a wavefront of electrical activity leaving the region of decreased conduction velocity. This slow foot is characteristic of a decremented action potential which has crossed the N zone and produces a response in the adjacent tissue (13, 14). In addition, the N region of the AV ring exhibits different rising velocities in the same cell during normal and retrograde stimulation. Data obtained from numerous cells in the AV nodal region of the adult rabbit heart indicate a decrease in the rising velocity of action potentials which is related to the distance crossed by the wave of excitation (14). Since the present findings are in agreement with those of the earlier adult heart studies, it may be inferred that a decrementing region is present in the embryonic chick AV ring comparable in function to that of the adult rabbit AV node.

Additional support for this proposal is evident as a result of rapid atrial excitation of the embryonic chick heart. At frequencies which do not significantly alter the shape of either the atrial or ventricular action potentials, steplike potentials of decreased amplitude are recorded from the cells of the middle and upper AV ring. AV block is observed concomitantly with the presence of a diminished, slowly rising action potential in the AV ring cells. This finding is illustrative of the fact that during high frequency stimulation,

AV transmission fails without any apparent involvement of the refractory period; the resultant effect therefore is attributable to the augmented conduction decrement.

C. *The Response to Acetylcholine and Norepinephrine*

Electrogram recordings reveal that both acetylcholine and norepinephrine affect the heart rate of the 10 to 13 day embryonic chick in a manner comparable to earlier findings (5, 11). However, systematic studies relating the effects of acetylcholine and norepinephrine on embryonic AV transmission have not been reported in the literature. The embryonic AV ring is now known to be responsive to both substances. The present results are similar to those reported for the adult mammalian heart (2, 16). The mechanism of the AV blocking effect of acetylcholine has been shown in the rabbit to be a depression and abolition of the slow AV nodal action potential (3, 7, 13). Norepinephrine has been reported to enhance AV propagation by affecting both the mammalian AV nodal region and His bundle (16). The accumulation of the various results in the present study is in support of the view that homologous structures in the embryonic and adult hearts are capable of responding similarly to comparable environmental stimuli.

In conclusion, a relationship between the embryonic chick AV ring and adult mammalian AV node may be established upon several lines of evidence: (*a*) membrane potentials recorded from both structures are similar in configuration, magnitude, and response time to propagated activity, (*b*) action potential shapes recorded from the adult mammalian AV ring are similar to those of the AN region of both embryonic AV ring and adult mammalian AV node, (*c*) the wave of excitation crosses the AV ring and AV node in a direction perpendicular to the AV valve, (*d*) the delay in AV transmission can be localized and characterized, in both structures, to the cells of the N region, (*e*) the response of both embryonic AV ring and adult AV node to forward and retrograde propagation is comparable, and (*f*) both structures respond similarly to rapid atrial excitation, acetylcholine, and norepinephrine. It has been well established that the electrocardiogram of the embryonic heart shows a normal PR interval at a stage of development in which muscular continuity is maintained along the entire AV junction (1, 6, 18, 21). The results of the present investigation suggest that the delay in transmission is attributable to the AV ring tissue, which is, in essence, the functional counterpart of the adult AV node.

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