Correlative Electrophysiological and Anatomical Studies Concerning the Site of Origin of Escape Rhythm during Complete Atrioventricular Block in the Dog

THOMAS N. JAMES, JAMES H. ISOBÉ, AND FERDINAND URTHALER

SUMMARY Complete heart block was produced in eight dogs by the selective perfusion of physostigmine or neostigmine into the atrioventricular (AV) node artery. A characteristic escape AV junctional rhythm emerged in each dog. After reversal of the cholinesterase paralysis with atropine, in each dog partial heart block was produced by an incision into the AV nodal region. In three of these eight dogs, a second incision placed slightly more anteriorly produced complete AV block which was followed by the emergence of an escape AV junctional rhythm similar to the one produced pharmacologically. Hearts of these three dogs were examined histologically with serial sections to determine the exact location of the incisions and their relationship to the AV node and His bundle. In each dog the incision that produced complete heart block passed directly through the junction of AV node with His bundle. In this region previous studies had demonstrated numerous P cells, which are thought to be the site of origin of normal cardiac automaticity. In each of the three hearts there were abundant P cells in continuity with the His bundle distal to the cut producing heart block. Significance of these findings is discussed relative to the locus of action of acetylcholine within the AV junction, the site of origin of AV junctional rhythm, and some aspects of the experimental and therapeutic production of heart block.

Ore Res 45:108-119, 1979

WITHIN the sinus node there is a kind of cell that has been postulated to be normally automatic, and both physiological and anatomical evidence supports the hypothesis (Trautwein and Uchizono, 1963; Woods et al., 1976; Taylor et al., 1978). These special cells are named P cells (James et al., 1966). They occur most abundantly in the sinus node or near the junction of the atrioventricular (AV) node and His bundle (James and Sherf, 1968), although some have been observed in the Eustachian ridge or Bachmann's bundle (James and Sherf, 1972) and in the AV ring near the mitral valve of a human subject who died with the Wolff-Parkinson-White syndrome (James and Puech, 1974). The cytology and histological organization of groups of P cells are similar in canine (James, 1964) and human (James, 1961) hearts.

In recent experimental studies we have found two forms of AV junctional escape rhythms, the rates of which bear a distinct mathematical relationship to each other and to the rate of the control sinus rhythm of the same canine heart (Urthaler et al., 1973; Urthaler et al., 1974). The first of these is named AVJ-1 rhythm and consistently emerges as the dominant cardiac rhythm if the sinus node is selectively suppressed by the administration of appropriate agents perfused directly into the sinus node artery (Urthaler et al., 1973). AVJ-1 rhythm responds normally to selectively administered acetylcholine or to vagal nerve stimulation just as does the sinus node. AVJ-2 rhythm consistently emerges when the AV node and His bundle region are selectively perfused with physostigmine or neostigmine via the cannulated AV node artery (Urthaler et al., 1974). The emergence of AVJ-2 rhythm is preceded in such experiments by the progressive development of complete AV block. It will respond to norepinephrine but not at all to acetylcholine, can be suppressed by overdriving, and has all the electrocardiographic and electrographic features of a supraventricular rhythm. In several hundred such canine experiments, we have never seen the emergence of any other stable automatic rhythm.

The present studies were conducted to compare the electrophysiological effects of either pharmacological (physostigmine) or surgical heart block with the exact histological location of the incision which produced heart block. As a corollary, we also consider the nature of the escape rhythm after the heart block (produced by either method) and its possible site of origin.

Methods

Eight mongrel dogs of either sex were anesthetized with sodium pentobarbital, 30 mg/kg, iv, and
then were intubated for mechanical ventilation with room air. After the chest was opened in the midline, epicardial electrodes were placed, and a bipolar recording catheter was wedged in the aortic root for recording electrograms from the vicinity of the His bundle. Details of these procedures, including this method for recording His electrograms, have been published previously (Urthaler and James, 1975).

Electrograms from the aortic root exhibit a remarkably similar form and an identical location of the His potential as compared with a Hoffman-type multipolar electrode sewn directly over the AV node and His bundle; furthermore, this similarity persists during all degrees of selectively produced heart block and during a variety of arrhythmias (Urthaler and James, 1975). Multiple surface ECG leads were recorded, and one then was selected as representative to illustrate. For AVJ-1 and AVJ-2 rhythms, the importance of multiple surface ECG leads has been previously appreciated (Urthaler et al., 1973; Urthaler et al., 1974). Small cannulae were inserted into either the AV node artery or sinus node artery for their selective perfusion (James et al., 1970; James and Nadeau, 1962; Urthaler and James, 1977). Cloth tapes were placed around the superior and inferior venae cavae near the right atrium to permit brief caval occlusion, during which the right atrium was opened quickly to expose the region of the AV node and His bundle. Gross and microscopic anatomy of this region has been the subject of previous reports from this laboratory (James, 1964; James et al., 1970).

AVJ-1 rhythm was produced as previously described (Urthaler et al., 1973) but did not prove relevant to other results, for reasons to be discussed later. To produce AVJ-2 rhythm in each dog, either neostigmine hydrochloride or physostigmine salicylate (100 μg/ml) was perfused into the AV node artery as reported previously (Urthaler et al., 1974). Complete AV block was gradually established over a period of about 1 minute, following which a typical AVJ-2 rhythm emerged. This slow but regular supraventricular rhythm could be overdriven readily by ventricular pacing, after which there was the characteristic warm-up period seen with automatic rhythms. QRS complexes were narrow and ventricular electrograms were preceded by a His potential. Once the nature of this AVJ-2 rhythm was demonstrated, the local effect of cholinesterase paralysis was abolished by the selective administration of 10 μg of atropine into the AV node artery. All features of the electrocardiogram and local electrograms

**Figure 1** A control record for dog no. 1 is compared with one soon after the administration of physostigmine (eserine) into the AV node artery (AVNA). In the latter record, a transition from partial to higher grade AV block is shown. Several seconds later, complete AV block was established and sustained (not shown here). Channels from above down include central aortic pressure (Ao) scaled in mm Hg, a His bipolar electrogram (HBE), bipolar electrograms from sinus node region (SN) from right ventricle (RV), and from left ventricle (LV), and a surface lead (aVR). Recording paper speed here and in all subsequent polygraphs is indicated by a horizontal time-reference bar.
then were identical to the control period, including intervals between the sequence of all components in each local electrogram.

When the pharmacological control observations were complete and conditions were stable and identical to the initial control period, the right atrium was quickly cut open during brief caval inflow occlusion. Since His electrograms were being recorded from the aortic root, no prior injury of any type was inflicted on or near the AV node or His bundle, as might have been the case with a locally placed right atrial catheter electrode or a multipolar plaque sewn there; furthermore, the region was not encumbered by such devices, leaving the site free for the cuts. There were two goals for incisions to be placed in the AV junction. The first was to attempt a cut which would pass above and completely around the AV node, anticipating that this would interrupt all sinus signals and permit the escape of AVJ-1 type rhythm (Urthaler et al., 1973). The location of this elliptical cut was judged on the basis of previous dissection and histological examinations. This procedure was attempted during brief caval occlusion in seven of the eight dogs, and it was never possible to produce complete AV block or to interrupt completely the delivery of sinus impulses into the AV junctional region. In every dog, partial AV block could be readily produced, varying from prolongation of P-R and A-H intervals to 2:1 and 4:1 AV block, but in each of these there was steady improvement in the facility of AV conduction, probably attributable to recovery from some of the acute injury associated with the cut.

The second surgical incision was intended to produce complete AV block of the type seen with selective perfusion of either neostigmine or physostigmine into the AV node artery. When the first incisions failed to produce AVJ-1 rhythm, the next incision was placed a few millimeters more anterior within the junction of atrial and ventricular septa, just above the septal leaf of the tricuspid valve. This regularly increased the degree of block and, in two of seven dogs, was followed by the prompt appearance of sustained complete AV block and the emergence of AVJ-2 rhythm, as will be described later. In the five other dogs, repeated incisions of this type either failed to produce sustained complete AV block, or the aorta or left ventricle was entered by the incision, and bleeding could not be controlled without variable amounts of ischemic or mechanical injury in the AV node or His bundle. Based on our experience with the preceding seven dogs, in the eighth dog we placed a single successful incision vertical to the septal junction and promptly produced sustained complete AV block with AVJ-2 escape rhythm.

The hearts of all three dogs in which an incision had produced complete AV block and escape AVJ-2 rhythm were later removed and fixed in formalin for histological examination. The region of the AV junction was excised in a single block and oriented in a manner to permit serial sections in a plane parallel to the two AV valve rings. This plane is perpendicular to the general plane of two septa and is particularly suitable for demonstrating the longitudinal anatomy of the AV node and His bundle with its proximal branches (Massing and James,
AV BLOCK AND AV JUNCTIONAL ESCAPE RHYTHM
James et al. 1976). Furthermore, it was ideal to delineate the exact anatomical relationship between the incisions, the AV node and the His bundle. Serial sections were cut at 8-μm intervals, every 10th section being saved. Slides were prepared routinely with the Goldner trichrome stain. More than 2000 sections were prepared from each of these three hearts.

Results

Dog No. 1.

Administration of physostigmine into the AV node artery of this dog led to the characteristic progressive increase in AV block and ultimately to an AVJ-2 type escape rhythm (Fig. 1). After complete AV block was established, there was slight variation in the QRS configuration, but a His complex always preceded the ventricular complex. Once the AVJ-2 rhythm stabilized, there was no significant variation in its rate even though QRS configuration did vary. The additional administration of acetylcholine directly into the AV node artery did not alter either the degree of AV block or any aspect of the AVJ-2 escape rhythm. Atropine was then administered into the AV node artery and promptly restored control sinus rhythm with normal AV conduction.

During caval occlusion, we then opened the right atrium and placed one elliptical incision in the region just above the AV node, hoping to cause an AVJ-1 type escape rhythm by interrupting all input to the crest of the AV node. Only incomplete AV block was produced. A second incision then was made just anterior to the first one in an effort to cut just between the AV node and His bundle. This was successful in producing complete AV block with typical AVJ-2 escape rhythm (Fig. 2). During the initial period of observation after the incision had produced complete heart block, the QRS configuration varied exactly the way it had during complete AV block produced by physostigmine. With the surgical heart block, some small new deflections appeared, following the atrial complex in the His bundle electrogram (Fig. 2); these were consistent in location and appearance. Among other possibilities (including local ordinary atrial activity), these may represent His potentials that were totally separate or dissociated from the other His potential regularly preceding the ventricular complexes. Such "atrial" His potentials had not been present in the control observations or during the pharmacology.
cally produced heart block. The administration of acetylcholine selectively into the AV node artery after the surgical production of heart block caused transient atrial fibrillation, as such perfusion often does, but there was no influence on either the degree of heart block or the rate of the AVJ-2 rhythm or configuration of electrocardiographic complexes (Fig. 2).

Stable AVJ-2 rhythm continued for over 10 minutes, at the end of which time the heart was removed quickly and prepared for histological study. Both surgical incisions were readily identified grossly and histologically. The first incision extended only into the proximal margin of the AV node but did not transect it (Figs. 3 and 4). The second incision cut into the junction of AV node with the His bundle and completely transected these structures by extending to the central fibrous body (Figs. 3-5). P cells were present on both sides of the second cut (Fig. 5). Typical slender interweaving transitional cells comprised the AV node proximal to these P cells, and longitudinally oriented Purkinje fibers extended forward from similar P cells in forming the His bundle. Distribution of these characteristic histological features and the extent of each cut were carefully delineated from serial sections of the region.

**Dog No. 2**

Pharmacological production of complete AV block and escape AVJ-2 rhythm was achieved readily with physostigmine selectively perfused into the AV node artery. Prior to the physostigmine, a similar response (complete AV block and AVJ-2...
rhythm) was produced transiently in this same dog by stimulating the intrathoracic portion of the right vagus nerve.

During caval occlusion, a first incision was intended to separate the AV node from the sinus impulse input, but again failed, although the P-R interval was prolonged. The second incision, then placed as in dog no. 1, was successful in producing complete AV block and escape AVJ-2 rhythm. QRS configuration during AVJ-2 rhythm in this dog eventually was identical during the complete AV block produced either by physostigmine or by the cut, and was the same as during sinus rhythm and normal AV conduction in the control period. However, during the initial phase of AVJ-2 rhythm, the QRS configuration varied from the control, but this same variation occurred whether complete block had just been produced by vagal stimulation, by physostigmine, or by a cut. Stable complete AV block and AVJ-2 rhythm were again observed for 10 minutes, and the heart then was removed for histological examination.

The first incision had been predominantly into the interatrial septum and had involved only a small margin of the AV node. The second incision cut directly through the junction of AV node and His bundle and transected the region completely. P cells of the nodal-His junction were present distal to the cut and continuous with the Purkinje fibers of the His bundle.

**Dog No. 3**

Selective pharmacological production of complete AV block and AVJ-2 rhythm was achieved with physostigmine administered into the AV node artery as in the other dogs. After the cholinesterase block was reversed with atropine and control rhythm and conduction reestablished, the right atrium was opened during brief caval occlusion. This time a single incision was made, and complete AV block and escape AVJ-2 rhythm were produced. To confirm the degree of AV block and the nature of the escape rhythm, transient atrial fibrillation was produced on two occasions by selective perfusion of acetylcholine into the cannulated sinus node artery. Both the rate of the AVJ-2 rhythm and the
configuration of all ventricular complexes remained the same before, during, and after this transient atrial fibrillation. Stable complete AV block and escape AVJ-2 rhythm persisted for over 20 minutes of observation, and then the heart was removed for histological examination (Fig. 6).

The single incision in dog no. 3 went directly through the junction of the AV node and His bundle (Figs. 7 and 8). P cells were present distal to the cut, continuing with the His bundle beyond that point (Figs. 9 and 10). A few P cells were also intermingled with the slender interweaving transitional cells of the AV node proximal to the cut (Fig. 10).

**Discussion**

In each of the three successful experiments, both the complete AV block and the escape AVJ-2 rhythm were similar, whether produced by selective perfusion of physostigmine into the AV node artery or by cutting the junction of the AV node and His bundle. Furthermore, the successful surgical incision was identically located in each heart, passing directly through P cells of the nodal-His junction but always leaving some P cells connected to the His bundle. The anatomical findings thus support the concept that AVJ-2 rhythm originates from P cells in the His bundle region.

Electrophysiological similarity of both the pharmacological and the surgical results suggests that the site of action of acetylcholine on the AV junctional region closely corresponds to the site of the successful surgical incisions. We have postulated previously that the P cells are directly influenced by acetylcholine to produce both negative chronotropic and negative dromotropic effects (James, 1976; James et al., 1970; James and Sherf, 1968; Urthaler and James, 1977). It is known further that acetylcholine has little or no effect on conduction velocity in Purkinje fibers (Hoffman and Cranefield, 1960). These observations collectively suggest that acetylcholine acts most effectively on those myocytes having the fewest and smallest gap junctions (i.e., junctions between P cells) and is least effective when there are numerous large gap junctions (Purkinje cells).

Others have suggested that the His potential must arise from the proximal portion of the His bundle (Kupersmith et al., 1973). At that location the groups of P cells closest to the His bundle make their terminal connection to the Purkinje fibers of the His bundle via transitional cells, and all these

---

**Figure 6** In dog no. 3, a single cut produced complete heart block with escape AVJ-2 rhythm, as illustrated in these two records. QRS complexes are similar in both records. Physostigmine in the AV node artery of this dog produced exactly the same results as this cut.
junctions include increasing numbers and sizes of gap junctions (James and Sherf, 1968; James and Sherf, 1971). This could explain the irrepressibility of AVJ-2 rhythm by even enormous amounts of acetylcholine (Urthaler et al., 1974), since the last few potential sites of automaticity (P cells) would have increasing numbers of intercellular connections (gap junctions) not responsive to acetylcholine. However, this presupposes that impulse formation by P cells is less responsive to acetylcholine than conduction from them would be, or that the chronotropic responsiveness has some minimum level below which it cannot be depressed further, even by very large amounts of acetylcholine.

There are two additional reasons for suspecting that both AVJ-2 rhythm and the His potential originate within the P cells of the nodal-His junction. One is the fact that local selective chelation of calcium ion within the AV junction (and presumed dehiscence of intercellular connections deficient in gap junctions, meaning especially between P cells) causes reversible splitting of the His potential (James, 1976). The second reason is the apparent splitting of the His potential in the present experiments in dog no. 1 by an incision which cut directly through the P cells of the nodal-His junction (Fig. 2). Considering the variability of the plane of these incisions and consequent unpredictability of the magnitude or exact direction of any electrical vector for which the divided P cell groups might be responsible, it is not surprising that what is thought to be an atrial component of the split His potential was not distinctive in the other two dogs.

As for the variability of QRS configuration and duration, stable rhythm with either form of QRS was not associated with any significant change in rate. Furthermore, the QRS variability was similar, whether AVJ-2 rhythm was caused by physostigmine or by surgical cutting. We believe that these observations are collectively best explained by some
intermittency in the exact signal front generated by the same group of P cells or by intermittent variation in the pattern of conduction within the longitudinally partitioned His bundle. Evidence has previously been presented as to why we believe that longitudinal partitioning of the His bundle would influence normal as well as abnormal conduction there (James and Sherf, 1971; Sherf and James, 1972; Sherf and James, 1969). Recent observations (Fabregas et al., 1976; Narula, 1977) as well as some older ones (Sciacca and Sangiorgi, 1957) by other investigators support this suggestion. This explanation for QRS variation remains hypothetical, however, because local microelectrode recordings were not obtained.

Since AVJ-2 rhythm in both our pharmacological and surgical experiments exhibited overdrive suppression and warmup, we take this as evidence that it was automatic and not reentrant in nature. To the present time, we have not been able to produce incisions into the distal His bundle or completely transect the proximal left bundle branch without also producing inordinate bleeding from the root of the aorta or from the left ventricle, control of which requires additional mechanical manipulation in the region and an unpredictable but probably large amount of additional mechanical injury there. Simple inspection of the anatomical proximity of the crucial structures involved (His bundle or its proximal branches, cavity of left ventricle, root of aorta, as seen in Figs. 3 and 7) illustrates the formidable task. It also helps explain why some examples of "surgical heart block" have been followed eventually by recovery of AV conduction, even at long periods after the event (Reid et al., 1976).

There is some normal anatomical variability of the location, concentration, and histological organization of P cells and their neighbors within the AV junction. This variability is comparable in human and canine hearts (James, 1961; James, 1964; James and Sherf, 1968). In general, most P cells are clustered deep within the AV node near its junction with the His bundle. However, there are scattered P cells within the body of the AV node and a smaller number within some portions of the His bundle. Even the number and organization of P cell

**FIGURE 8** In dog no. 3, the single cut went completely through the distal portion of AV node (A) and the proximal portion of His bundle (B), the forward continuity of which is indicated with open arrows.
clusters at the nodal-His junction differ from one heart to another. Considering this anatomical variability, it is to be expected that the physiological consequences of cutting into the region may have slightly different effects in one dog compared to another, and these consequences probably include variability of QRS complexes when the P cell groups are located more eccentrically.

Although it was not possible in these experiments on the intact heart in vivo to investigate the resting and action potentials of individual cells in the region of AV node and His bundle, it should be noted that some studies in vitro have failed to demonstrate any spontaneous automaticity in the AV node (Hoffman and Cranefield, 1964). It is possible that P cells in this region are too deeply placed to be readily accessible for impalement with a microelectrode. Furthermore, studies with microelectrodes of impulse generation by Purkinje fibers in vitro have demonstrated that acetylcholine does cause changes in the transmembrane potential (Danilo et al., 1978; Tse et al., 1976). However, although we believe that further efforts are merited to try to correlate anatomical observations with such experiments with microelectrodes, there are some important constraints in what can ultimately be learned with the single cell records. It now seems probable that most electrophysiological properties of the AV junction are necessarily multicellular functions. Knowing everything about one or a few cells might not only be incomplete but even incorrect information about the way many such cells would behave in the aggregate. Local electrograms and light microscopy would thus offer distinct advantages that microelectrodes and electron microscopy cannot.

Because of the remarkable similarity of all the electrophysiological characteristics of heart block and escape AV junctional rhythm, whether produced pharmacologically or surgically in our experiments, there are at least five reasons why the pharmacological method may be preferred for the experimental purpose. First, one can achieve the same results without atriotomy, which is necessary for surgery under direct vision; other forms of incision (e.g., blind) are grossly inaccurate as to location.

![Figure 9](http://circres.ahajournals.org/)

**Figure 9** More details of the cytology bordering the cut in dog no. 3 are shown here. Many P cells are seen at the proximal margin of the His bundle (A), with the cut going directly through that region. Purkinje fibers leaving that area to course anteriorly become progressively more longitudinal in their orientation. Some of the P cells near the Purkinje fibers are boxed in A and shown at higher magnification in B, where several P cells are encircled.
Second, the procedure of cannulating the AV node artery for its selective perfusion is associated with no electrophysiological or hemodynamic perturbation of significance (James et al., 1970; Urthaler and James, 1977). Third, pharmacologically produced heart block and escape AV junctional rhythm are completely reversible and repeatedly reproducible in the same dog and are comparable in all dogs.

**Figure 10** P cells on either side of the cut in dog no. 3 are shown in detail here. Just beyond the cut, a group of P cells is seen mingling with longitudinally orienting Purkinje fibers of the His bundle in A. P cells of the AV node are deeply placed relative to the endocardium, but some are distinctly seen proximal to the cut (B).
**AV BLOCK AND AV JUNCTIONAL ESCAPE RHYTHM/James et al.**

**Fourth,** the results with pharmacological production of heart block are totally predictable, particularly when compared to results from crude procedures, such as needle injections of alcohol or formaldehyde into the AV node region, and more selective than the results from a broader area of injury necessarily attendant to cauterization or freezing of the AV nodal region. **Fifth,** selective perfusion through the AV node artery may be combined with the placement of local electrodes directly over or into the region of the AV node and His bundle, procedures which are impossible with surgical, cautery, or freeze injury in those structures.

Finally, there may be some applicability of our findings to the growing number of efforts in surgical treatment of intractable arrhythmias in human subjects (Sealy et al., 1977). Many such procedures are based on incisions made into the region of the human AV node and His bundle with the intention of creating permanent heart block. When successful, surgical heart block should be permanent and may be the desirable or only means of terminating life-threatening arrhythmias. On the other hand, if such a heart block does not terminate the arrhythmias, it cannot be reversed. It would be useful to predict the outcome of such surgery more accurately than most present methods permit. For example, it should be feasible to administer acetylcholine or neostigmine selectively into the AV node artery under angiographic control or even with direct visualization of the appropriate coronary artery during surgery. It would be necessary that an example of the arrhythmia be present or be produced to be tested. The effects of acetylcholine are very transient (seconds), whereas those of neostigmine last much longer, but at any time are immediately and completely reversible with atropine. The cholinergic tests could be repeated as many times as necessary to establish an answer to the question. Some arrhythmias might even be terminated successfully by a single injection of acetylcholine or neostigmine and then not recur, although it is likely that most would recur. There is unfortunately no way to produce comparable degrees of neostigmine effect on the AV node and His bundle by its intravenous administration because of the numerous intolerable extracardiac effects it would produce. However, if the heart block from neostigmine in the AV node artery did indeed terminate the arrhythmia, one could more accurately and confidently predict the therapeutic effectiveness of surgically produced heart block.

**References**


Reid JM, Coleman EN, Doig W (1976) Reversion to sinus rhythm 11 years after surgically induced heart block. Br Heart J 38: 1217-1219


Correlative electrophysiological and anatomical studies concerning the site of origin of escape rhythm during complete atrioventricular block in the dog.
T N James, J H Isobe and F Uthaler

Circ Res. 1979;45:108-119
doi: 10.1161/01.RES.45.1.108

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/45/1/108

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://cirres.ahajournals.org/subscriptions/