Response of the A-V Node of the Rabbit to Stimulation of Intracardiac Cholinergic Nerves

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ABSTRACT

The cholinergic innervation of the rabbit heart was studied in vitro and in vivo. An isolated preparation, which included the combined atria, S-A node, A-V node and bundle of His, was mounted so that microelectrodes could be placed in either the specialized or nonspecialized tissues. Using a roving monopolar electrode, local transmural electrical stimulation of intracardiac nerves (nerve stimulation) was applied at the A-V or the S-A node. At the A-V node it induced hyperpolarization, reduced the duration and amplitude of action potentials recorded from three regions of the A-V node and the His bundle, whether the preparation was spontaneously beating or electrically driven in either the forward or retrograde direction, and blocked membrane excitation in the nodal and nodal-His regions and to downstream structures. The depressant effects of nerve stimulation were augmented by physostigmine and antagonized by atropine. Addition of acetylcholine to the bath reduced the frequency at which the A-V node conducted, but in the concentrations used did not duplicate the effects of nerve stimulation. The behavior of the S-A node is qualitatively similar to that of the A-V node. In the presence of a sinus rhythm, nerve stimulation applied at the A-V node did not affect the activity of the S-A node, and during artificial atrial drive, nerve stimulation of the S-A node did not affect A-V nodal function, indicating that little or no neural communication exists between the two regions in the isolated preparation. Locally released acetylcholine appears to depress excitability of specialized cells in the central node, independently of the direction of propagation.

ADDITIONAL KEY WORDS transmembrane potentials S-A node cholinergic neurotransmitter innervation in specialized atrial tissues conduction block driving frequency and A-V conduction rabbits

For many years attention has been directed to the influence of A-V nodal conduction on cardiac performance. The introduction of intracellular recording methods has contributed much to the investigation of A-V nodal function. From such studies Hoffman et al. (1-5) proposed that a characteristic feature of the A-V node is the phenomenon of decremental conduction, and emphasized the functional changes which occur in the isolated tissue during rapid atrial excitation combined with the application of acetylcholine. The major change in the A-V node associated with increased frequency of atrial excitation was shown to be a progressive decrease in the rate of depolarization and in the amplitude of nodal action potentials (3). Atrial excitation at a frequency higher than 6.2/sec was not followed by excitation of the A-V node (6). The application of acetylcholine caused marked depression of the amplitude of nodal action potentials during forward, but not retrograde, conduction (2, 5). The observed decrease in the duration and amplitude of atrial action potentials was regarded as an important determinant for the cholinergic block of A-V conduction (5). It was proposed that cholinergic changes in atrial fibers induced consequent alterations in the rate and extent of depolarization in cells at the atrio-nodal junc-
tion, and that this decrement in depolarization resulted in decremental conduction within the node (2, 5).

It was demonstrated in this laboratory that selective stimulation of cholinergic endings in the A-V node can induce local cholinergic effects (7), but further analysis was not undertaken. Local electrical stimulation with pulses of short duration, applied to the S-A node, has been shown to release neurotransmitters from postganglionic (8), cholinergic and adrenergic terminals and to induce chronotropic and inotropic functional changes. The effects have been shown to be similar to those resulting from preganglionic stimulation of the extracardiac vagus (9). The availability of these methods for application to the isolated A-V node preparation encouraged us to examine the response of the A-V node to the local application of the cholinergic neurotransmitter. This investigation will attempt to answer these questions: (A) What membrane changes occur in the A-V node in response to local nerve stimulation? Will they help to delimit functional regions of the node? (B) Will the response of the A-V node to endogenous acetylcholine depend on the direction of conduction or on changes in adjacent nonnodal structures? (C) If relatively weak electrical pulses applied artificially cause the release of intranodal neurotransmitters, will the rapid depolarization of cardiac tissue also induce release? (D) What neural communication exists between the S-A and A-V nodes? Between the nodes and the atrial myocardium? (E) How do the A-V and S-A nodes compare in terms of their response to nerve stimulation?

Methods

Forty-one albino rabbits of either sex were used. Those providing tissue for isolated atrial preparations ranged from 1.8 to 2.2 kg, whereas those used for in situ experiments ranged from 2.0 to 2.5 kg.

Isolated Tissue Preparation

The entire heart was removed from rabbits which were anesthetized with ether. The heart was temporarily immersed in a modified Ringer solution warmed to 30° C and gassed with 95% O₂-5% CO₂. Most of the ventricular mass and extraneous adherent tissues were dissected free and discarded. The specimens retained for experimental use included the S-A node, the combined atria, the A-V node, the His bundle and bits of the ventricles. The anterior wall of the superior vena cava was opened following the method of Paes de Carvalho et al. (10) and secured to a pair of stainless steel hooks on the arm of a Grass FT 03 force transducer. Ventricular tissue near the A-V node was pinned to the edge of the chamber, and the right atrial appendage was immobilized by fixing it on a pair of stimulating electrodes. The arrangement is diagrammed in Figure 1. The experimental chamber was a 60-ml Flexiglas bath maintained at 30 ± 0.5° C, through which buffered oxygenated Ringer solution flowed at a rate of 3 to 5 ml/min. The composition of the nutrient solution was (in mM): Na, 162.1; K, 5.4; Ca, 2.2; Cl, 157.0; HCO₃, 14.9; and dextrose, 5.6. After mounting the preparation a 60- to 90-min equilibration period was allowed before experimental procedures were begun.

Extracellular and intracellular electrical activity were recorded. Atrial activity recorded from an extracellular bipolar electrode served as a time reference point. Transmembrane potentials from various parts of the A-V node, His bundle, S-A node and the atrial myocardium were recorded, when desired, with a floating microelectrode. Further details on the sites of recording in the A-V node are given in the section on Results. Transmembrane potentials were analyzed in terms of their response to nerve stimulation.

FIGURE 1

Diagram of the preparation of rabbit heart. 1, A-V node; 2, bundle of His; 3, S-A node; 4, musculi pectinati of right atrium; 5, crista terminalis; 6, superior vena cava; 7, inferior vena cava; 8, orifice of coronary sinus; 9, left atrium; 10, tricuspid valve. Solid circles = position of electrode for stimulating intracardiac nerve fibers: A, A-V nodal stimulation; B, S-A nodal stimulation. Solid triangles = position of electrode for driving muscle fibers: a, atrial drive (forward to A-V node and retrograde to S-A node); b, S-A nodal; c, His bundle (retrograde to A-V node). Pairs of arrows = positions at which the preparation was fixed.
of changes in configuration, amplitude, course of repolarization and voltage changes during dias-
tole. The contractile force of the isolated prepara-
tion was represented by the tension recorded be-
tween the portions shown in Figure 1, except in
those experiments in which it was desired to ob-
serve the contractile force of either the right or
left atrium following the application of local
stimulation to nodal tissue. Recordings were dis-
played on a Tektronix 502 oscilloscope or on a
Sanborn model 150 four-channel oscillograph.
A monopolar silver electrode 0.5 mm in di-
ameter and insulated to the tip was used for
transmural electrical stimulation of intracardiac
cholinergic and adrenergic nerve fibers innervat-
ing the S-A node, A-V node and atria. This
method of local stimulation is the electorelease
procedure reported previously (7) but will be
termed “nerve stimulation” in the remainder of
this report. Except as noted, nerve stimulation is
the local application of pulses 0.1 msec in dura-
tion and about twice the threshold voltage of the
nerve, applied at a frequency of 100/sec for 1
sec. The intensity of the nerve stimulation was be-
low the threshold for myocardial excitation. In
Figure 1 the optimal electrode positions for choli-
nergic nerve stimulation are shown as solid cir-
cles.
To provide electrical drive of the preparation
a bipolar stimulating electrode was placed in one
of the positions shown as solid triangles in Figure
1. Stimulation was applied at interstimulus inter-
vals of 400, 320, 250, 200 or 160 msec, as de-
sired. The stimulus pulse duration was 1 msec
and the stimulation intensity was double the vol-
tage required to produce myocardial excitation.
Pulses were delivered from a Tektronix type 161
pulse generator. The interval was changed in
steps and controlled by a Tektronix type 162
waveform generator.
An important parameter in electrical record-
ings from the specialized tissue was the interval
between action potentials recorded from the sev-
eral areas. The frequency of discharge in these
recordings was expressed in terms of cycle dur-
tion. Changes in cycle duration reported for trans-
membrane action potentials are not to be inter-
preted as representative of the rhythmic activity
of the total preparation, but only of the area un-
der observation by microelectrode. Cycle duration
was measured during the control period and the
transitional period induced by cholinergic nerve
stimulation. Two measurements were used for the
analysis of change in cycle duration in response
to nerve stimulation; these are $I_1$ and $I_2$ (see Fig-
ure 2). $I_1$ is the interval between the final pre-
stimulation action potential and the first action
potential (not less than 50% of the amplitude of
the prestimulation potential) occurring either dur-
ing or following cholinergic nerve stimulation. $I_2$
is the interval between the final prestimulation ac-
tion potential and the first action potential at
which complete recovery from the stimulation was
shown to occur. $I_1$ can be looked upon as repre-
sentative of an absolute loss of excitability, where-
as $I_2$ represents a relative depression of excita-
ability.

IN SITU PREPARATION
Ten rabbits were anesthetized by ether and
maintained by the administration of pentobarbi-
tal sodium, 30 to 50 mg/kg, i.v. Under artifi-

![Figure 2](https://example.com/figure2.png)

**FIGURE 2**
Convention for measurement of cycle duration under steady state condition (SD) and in
response to nerve stimulation ($I_1$ and $I_2$) (see Methods). Nerve stimulation was applied
to the A-V node in the preparation electrically driven from the right atrium at a 320-msec interval.
Horizontal bar just under the tracing shows nerve stimulation applied. Upper tracing
= contractile force of atria; lower = membrane potential recorded from the nodal region of
the A-V node. Calibration for contractile force = 500 mg, and for membrane potential = 50 mV;
time scale = 500 msec.
cial respiration the chest was opened along the midline and silver bipolar recording electrodes were attached to the wall of the right atrium and the left ventricle. The right and left cervical vagi were cut, and small, shielded stimulating electrodes were placed on the peripheral stump of each nerve. Vagal stimulation was achieved by electrically stimulating either the right or the left vagus nerve with pulses 3 msec in duration applied at 20/sec for 5 sec, at about twice the threshold voltage. The experimental procedures were begun within 20 min and completed within 3 hours after thoracotomy. Recordings of cardiac electrical activity were displayed on a Sanborn model 150 two-channel penwriter.

**DRUG ADMINISTRATION**

For the in vitro experiments, drugs were added directly to the muscle bath, and the perfusion was stopped during the time of observation. It was shown by control experiments that the effect of stopping the perfusion was negligible within the experimental period. For the in situ experiments drugs were injected into the marginal ear vein. Acetylcholine bromide, physostigmine salicylate, atropine sulfate, and propranolol hydrochloride were used in the indicated concentrations or doses.

Data were prepared for presentation as means of the absolute values ± the standard error of the means. The significance of differences between means was estimated by Student's t test.

**Results**

I. ISOLATED TISSUE EXPERIMENTS

A. Nerve Stimulation and A-V Nodal Function

*Regions of the A-V node and their response to nerve stimulation*: The A-V node has been described by others (4) as consisting of three regions, defined on the bases of localization, configuration of the cellular action potentials and the extent of delay of conduction with reference to the S-A nodal potential. The three areas have been termed AN (atrio-nodal), N (nodal) and NH (nodal-His). Our preparations were suspended in a manner closely similar to that described by Carvalho (4). The three regions were then identified approximately by anatomical location and by the electrical characteristics said to typify the different types (4). The distinction between nodal action potentials and those recorded from either adjacent atrial or His bundle cells was clear (as judged by shape and timing), but within the area presumed to be the A-V node the distinctions between nodal cell types often were less definite. Recordings from cells termed AN in this report were always made in the proximal node (with respect to forward conduction), were represented by little diastolic depolarization, and continued to produce action potentials during nerve stimulation applied to the A-V node which was capable of blocking conduction through the node. Action potentials from the N region were recorded from the middle node, showed prominent diastolic depolarization and slow upstroke, and were abolished during local nerve stimulation. Action potentials representing NH cells were obtained from the distal node and generally resembled those from the N region, although the rate of diastolic depolarization was comparably less in NH recordings. Action potentials from the bundle of His were recorded from the proximal bundle, very near the area used for NH recordings, and usually were of greater amplitude and longer duration than the other potentials.

Figure 3 illustrates our recordings from cells of the three nodal regions, before and during nerve stimulation. The response of the AN region included a decrease in duration and amplitude of the action potential and an increase in the diastolic membrane potential (hyperpolarization). Typically, AN action potentials persisted in spite of A-V conduction block. In response to nerve stimulation the action potentials recorded from the N and NH regions were abolished or were severely depressed (Fig 4), and the amount of hyperpolarization was greater than in AN cells or cells of contiguous tissues. In the absence of cholinergic potentiating influences, complete recovery of membrane function occurred within a few seconds after the nerve stimulation was terminated. Although profound changes in the A-V nodal action potentials were produced by nerve stimulation at the A-V node, the electrical activity of the S-A node and the contractile force of the right atrium were not affected except in a few cases to be described later in this report. In the His bundle a prolongation of cycle duration, with little or no hyperpolarization, was induced in

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response to nerve stimulation of the A-V node. Only moderate, if any, change occurred in the configuration of the action potential (Figs. 3 and 5).

During forward conduction, nerve stimulation at the A-V node induced only slight changes in cycle duration in action potentials from AN cells, although hyperpolarization and a reduction in amplitude and duration were clearly evident. As previously described, a transient abolition of action potentials in the N and NH regions occurred with hyperpolarization, and His bundle potentials concomitantly disappeared without appreciable hyperpolarization. During retrograde conduction, action potentials in the N and NH regions again were abolished by nerve stimulation, although the activity of the AN cells persisted (with the membrane alterations induced by cholinergic influence) at the spontaneous frequency of the S-A node. These observations are interpreted to indicate that the block of A-V nodal conduction induced by local cholinergic nerve stimulation occurs within the nodal tissue between the AN region and the bundle of His, independently of the direction of conduction.

Recordings from the anatomical region presumed to contain N and NH cells were emphasized because the N region has been described as the site of decremental conduction in the A-V node (4) and because of the high sensitivity to cholinergic nerve stimulation of these sites. Several experimental conditions
NERVE STIMULATION IN THE A-V NODE

Typical changes of action potentials recorded from the N region in response to nerve stimulation (S) applied at the A-V node. Top, control; middle, just after the stimulation; bottom, 4 sec after. Upper tracing = extracellular recording from the right atrium; lower = membrane potential. Calibration for membrane potential = 50 mV; time scale = 100 msec.

Effect of frequency of nerve stimulation on the A-V nodal response: The relationship between the frequency of nerve stimulation and the response in the N region is shown graphically in Figure 6. The magnitude of the

Effect of frequency of A-V nodal nerve stimulation on membrane potential and cycle duration of action potentials recorded from the N regions in the spontaneously beating preparation. Upper graph: hyperpolarization. Lower graph: solid circle = steady state cycle duration; open = I_1; half = I_1. Vertical bars represent standard errors of means.

Changes in membrane potential and cycle duration from different regions of the A-V node (AN, N and NH), the His bundle (H) and the S-A node (SA) in response to nerve stimulation. Stimulation was applied at the A-V node for the first four, and at the S-A node for the last one. Upper panel: hyperpolarization. Lower panel: solid column = steady state cycle duration; hatched = I_1; open = I_1. Vertical bars represent standard errors of means.
hyperpolarization resulting from nerve stimulation (as shown in Figure 3) and the two intervals described under Methods and shown in Figure 2 were particularly sensitive to changes in the frequency of local stimulation. As indicated previously, an increase in the interval between action potentials represents the negative dromotropic response in the cells under observation. A similar intensity-frequency relationship has been reported for the negative chronotropic effect of nerve stimulation in the S-A node (11).

The electrically driven preparation: Since electrical pulses of insufficient intensity to excite the cardiac muscle fibers are capable of exciting intranodal cholinergic nerve fibers, it was postulated that cardiac electrical activity might cause neural excitation. If so, an increase in driving frequency would be expected to cause increased cholinergic depression of function. The preparation was driven electrically, either by stimulation of the right atrium (forward) or of the His bundle (retrograde). Five frequency steps were used (represented by intervals of 320, 250, 200, 160 and 130 msec) to determine the minimal intervals at which action potentials from the AN and N regions of the A-V node could respond with neither block nor decrement of excitability. In all cases a driving interval of 160 msec induced a 2:1 conduction block, regardless of the direction of propagation. In the AN region the minimal interval for forward excitation was 250 msec in 3 and 200 msec in 12 preparations. In the N region the interval was 250 msec in 7 and 200 msec in 13 preparations. For retrograde excitation in the AN cells the minimal interval was 250 msec in 2 and 200 msec in 4 preparations, whereas in the N region the minimal interval was 250 msec in 6 and 200 msec in 4 preparations.

During either forward or retrograde excitation, nerve stimulation was applied to the A-V node to assess the possible influence of driving frequency on cholinergic response. The responses of the N and NH regions to nerve stimulation are shown in Figure 7 for driving intervals of 320 and 250 msec. As the figure indicates, there was no evidence to sup-
Nerve stimulation in the A-V node

Effect of frequency of nerve stimulation applied at the A-V node on cycle duration from the N region in the presence of physostigmine in the spontaneously beating preparation. C = predrug control; P = 10^-4 g/ml of physostigmine. Solid circle = steady state cycle duration; open = I_1; half = I_2. Vertical bars represent standard errors of means.

The results were not conclusive although electrical drive at a rate higher than the spontaneous rate increased the negative dromotropic effect of nodal nerve stimulation, especially under the condition of retrograde conduction. From the data shown in Figure 8 it is not to be expected that driving rates of 4/sec would exert much effect on the response to cholinergic nerve stimulation. It is clear that tachysystoles representing frequencies as high as 10 to 20/sec (e.g., atrial fibrillation) might be necessary to determine whether rapidly firing myocardial tissue can release endogenous neurohormones within the A-V node.

Effects of cholinergic and anticholinergic drugs: Acetylcholine was administered directly into the muscle bath. Its influence on the minimal interval at which N or NH cells of the A-V node could respond to either forward or retrograde excitation is shown in Table 1. Although the results are variable, acetylcholine decreased the ability of the region observed to conduct as the driving rate was increased. The higher concentration (shown in Table 1) caused some shortening of the duration of the action potential and occasionally induced a step on the upstroke. However, concentrations as high as 2 × 10^-4
TABLE 1
Modification by Acetylcholine of the Minimal Interstimulus Interval Followed by the A-V Node in the Electrically Driven Preparation*

<table>
<thead>
<tr>
<th>Direction of excitation</th>
<th>Change in minimal interval</th>
<th>Number of preparations responding to acetylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 x 10^-2 g/ml†</td>
<td>7 x 10^-1 g/ml†</td>
</tr>
<tr>
<td>Forward</td>
<td>Increase</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Decrease</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>7</td>
</tr>
<tr>
<td>Retrograde</td>
<td>Increase</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Decrease</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>7</td>
</tr>
</tbody>
</table>

*Recording electrode in cells of the N and NH regions of the A-V node.
†Concentration of acetylcholine.

FIGURE 10
Typical responses to nerve stimulation at the A-V node of action potentials recorded from the N region of the A-V node (N) and from the bundle of His (H) in the presence of 10^-6 g/ml of physostigmine. Upper tracing of each chart = contractile force; lower = membrane potential. Continuous tracings interrupted for 10 sec in upper panel and for 13 sec in lower. Calibration for contractile force = 500 mg, and for membrane potential = 50 mV. Horizontal bars just under tracings show nerve stimulation.

g/ml did not produce the striking membrane changes typical of the response to nodal nerve stimulation.

The cholinergic responses to acetylcholine and to nerve stimulation were enhanced by physostigmine (Fig. 10) and blocked completely by atropine (10^-6 g/ml), although atropine alone did not alter the ability of the A-V node to respond to increasing frequencies of drive. In 2 of 9 preparations in which the cholinergic response to nerve stimulation was abolished by atropine, nerve stimulation of the A-V node caused a transient acceleration of the spontaneous rate, as shown in the upper panel of Figure 11. In panels B and C, from another preparation, the shift in timing of an NH potential is shown before and after nerve stimulation, indicating that the functional pacemaker had shifted nearer to the site of the recording electrode. The results
NERVE STIMULATION IN THE A-V NODE

B. Comparison of the S-A Node with the A-V Node

The ability of the cells of the S-A node to respond to an imposed drive was studied in the presence of atrial stimulation and during electrical drive by an electrode positioned in a portion of the S-A node in which no electrorelease occurred (Fig. 1, electrode b). Upon stimulation of the right atrium the minimal response interval of latent pacemaker cells near the crista terminalis was 200 to 250 msec, but was 250 to 400 msec for true pacemakers and for latent pacemakers previously described as Types 1 and 2 (12). The response of cycle duration and of diastolic membrane potential to S-A nodal nerve stimulation are shown in Figure 5 (spontaneous beat only) and Figure 7 (spontaneous beat and two different frequencies of atrial drive). The changes induced in the parameters observed were qualitatively comparable to those seen in the A-V node.

Under the condition of S-A nodal drive (therefore bypassing the junction between atrium and node), the responses of S-A nodal cells to nerve stimulation did not differ from those obtained during atrial drive.

In the spontaneously beating preparations, physostigmine (10^-a g/ml) increased I, but not I, of S-A nodal action potentials in response to nerve stimulation. This was interpreted to indicate escape by retrograde conduction of impulses arising in the A-V node, since it will be shown below that nerve stimulation at the S-A node did not cause the release of neurotransmitters in the A-V node.

C. Neural Communication in the Isolated Tissue Preparation

The experimental preparation offered an opportunity to determine whether efferent autonomic nerves pass through the S-A node to innervate the A-V node, or the reverse. It...
was thought that such information is of practical importance to laboratory investigators and might provide relevant generalizations. In spontaneously beating and in electrically driven preparations, evidence for remote effects of nerve stimulation was sought by recording from one node while applying nerve stimulation to the other. In the presence of an S-A nodal rhythm, cycle duration and membrane properties in S-A nodal cells were not changed when nerve stimulation was applied at the A-V node. However, in 2 of 24 preparations the isolated tissue was dominated by an A-V nodal pacemaker. In these two preparations nerve stimulation applied at the A-V node induced changes in the form of the S-A nodal action potentials, a shift of the pacemaker to the S-A node, and a slight reduction in spontaneous frequency (panel A of Fig. 12).

In preparations dominated by an S-A nodal pacemaker, nerve stimulation applied at the S-A node increased the A-V nodal cycle duration in all 7 preparations. In 3 of these the S-A nodal dominance of rhythm persisted during and after nerve stimulation (panel B of Fig. 11), but in the remaining 4 an A-V nodal rhythm arose during arrest of the S-A nodal pacemaker (panel C, Fig. 12). Hyperpolarization of A-V nodal cells was never induced as the result of nerve stimulation at the S-A node. When the preparation was driven electrically, either in the forward or the retrograde direction, no effects were detected in the A-V nodal cells upon nerve stimulation of the S-A node.

Atrial response to nerve stimulation applied at the nodes: To determine the propagation of nervous excitation into the atrial myocardium following the application of nerve stimulation to either node, the preparation was driven through the right atrium at a constant frequency slightly higher than the spontaneous frequency. Atrial contractile force was monitored. Nerve stimulation of the S-A node has been shown to cause negative inotropic effects (7) and changes in membrane potential (13) in atria of preparations treated in this way.

In the present study contractile force of the right atrium was not affected in 13 of 16 preparations when nerve stimulation was applied to the A-V node. In the remaining 3 preparations the contractile force was reduced by about 10%. Nerve stimulation at the S-A node reduced right atrial contractile force in all 5 preparations by 30 to 60% and the duration of the atrial action potential was reduced.

The contractile force of the left atrium was decreased by 10% in 1 of 3 preparations fol-
lowing A-V nodal nerve stimulation, and by 10% in 2 of 3 preparations after S-A nodal stimulation.

II. IN SITU EXPERIMENTS

It is a well-known fact that the specialized atrial tissues receive innervation from both the right and left vagi in higher mammals. However, unpublished results in this laboratory have shown for the isolated vagus-atrial preparation of the rabbit that stimulation of either vagus does not affect the electrical properties of the A-V node, although the S-A node responds with bradycardia and characteristic changes in membrane potential (12). From the results of local stimulation in the present study it is clear that cholinergic fibers to the A-V node do not pass through the S-A node or along the crista terminalis, nor do they ramify from the A-V node into the right and left atria. In the attempt to clarify the functional vagal innervation of the rabbit heart, the in situ preparation was used, as described in Methods.

In the anesthetized rabbit the mean cycle duration of the spontaneously beating heart was 250 ± 5 msec \((N = 28)\). Upon stimulation of either the right or left vagus the cycle duration was increased from 600 to 700 msec. The negative chronotropic response to stimulation of the right vagus did not differ significantly from that induced by left vagal stimulation. The atrioventricular delay was increased by 20 to 30% in 3 of 10 preparations after right vagal stimulation and in 5 of 9 preparations after stimulation of the left.

Upon the assumption that the simultaneous stimulation of sympathetic fibers in the cervical vagi might significantly antagonize cholinergic effects, propranolol \((50 \mu g/kg)\) was injected intravenously in 5 preparations. It was believed that propranolol in the dose used would be expected to exert little effect, not dependent on its specific action of beta-receptor blockade. Following the injection of propranolol the spontaneous cycle duration and the atrioventricular delay increased by 30 to 50% and stabilized at the new value. Under the influence of propranolol complete A-V block was produced in 2 of 5 preparations with right vagal stimulation, whereas complete block was produced in 4 of 5 preparations with left vagal stimulation.

Discussion

The changes which nodal nerve stimulation induces in A-V nodal action potentials and in the ability of the node to transmit excitation presumably are associated with the release of acetylcholine from cholinergic nerve terminals in the node. It was shown by Cranefield et al. (2) that exogenous acetylcholine produces characteristic changes in the action potentials recorded from the A-V node. Hoffman (5) suggested that the effect of acetylcholine on nodal cells is secondary to a primary decrease in the duration and amplitude of atrial depolarization in fibers just proximal to the atrio-nodal junction. This interpretation was supported by the observation that the application of acetylcholine in a concentration sufficient to cause severe depression of nodal action potentials during forward excitation did not alter action potentials in the same region in the presence of retrograde excitation (5). In our experiments the response of the A-V node to acetylcholine was variable, although an increase in the minimal interval for 1:1 conduction was directly related to the concentration, whether conduction was forward or retrograde. These observations, together with the results of nodal nerve stimulation, are sufficiently different from those noted above to deserve discussion.

Following cholinergic nerve stimulation applied at the A-V node, action potentials from the regions of the node described earlier in this report were characterized by appreciable hyperpolarization and changes in shape. In the regions designated as N and NH additional responses included either marked depression of amplitude or transient abolition of the recorded action potential. These changes can be explained as the result of an increase in the permeability of the membrane to potassium as has been shown in other supra-ventricular tissues [see review by Trautwein (14)]. The cellular consequences of cholinergic nerve stimulation were independent of the direction of impulse propagation and did not
depend on a concomitant change in the membrane voltage of atrial cells near the nodal boundary. The method of local nerve stimulation functionally identifies the regions termed N and NH as critical regions for cholinergic block of the A-V node following nerve stimulation. However, the amplitude and duration of depolarization of AN cells were depressed by nerve stimulation. In the case of forward conduction it is possible that this depression leads to decremental conduction in the N and NH cells, but since nerve stimulation produces the same effects in the N and NH cells during retrograde conduction the membrane effects of the endogenous acetylcholine on the nodal cells per se would seem to be basic to the conduction block.

Because the application of nerve stimulation in one node did not influence the function of the other (except as expected from the modification of the dominant pacemaker), it is apparent that no functional neural communication by peripheral cholinergic fibers exists between the S-A and A-V nodes in the preparation used. In the isolated preparation the right atrium appears to be innervated by fibers which pass through the S-A but not through the A-V node. The left atrium may be innervated by fibers passing through both nodes, although the demonstration of a functional innervation is less impressive than for the right atrium. The in situ experiments described show that both the S-A and the A-V nodes of the rabbit are innervated by both vagi. It is apparent that the isolation procedure necessarily involves interruption of the cholinergic innervation. The in situ experiments indicate a predominant innervation of the A-V node by the left vagus and a predominant innervation of the S-A node by the right vagus, but there is considerable overlapping between the distribution of the two vagi.

Although it can be hypothesized that impulses arising within the heart can cause the excitation of intracardiac neural structures, the evidence in this study for the hypothesis is meager. Because the degree of cholinergic influence resulting from the experimental application of subthreshold (for cardiac tissue) stimuli is directly related to the frequency, it was predicted that increased drive of the tissue adjacent to the A-V node (or the S-A node) would cause increased release of endogenous acetylcholine and that this would sum with the further release effected by nerve stimulation. Although the observed effect was slight, the further augmentation in the presence of physostigmine is considered of sufficient interest to merit inclusion in this report.

In the presence of a constant atrial drive, the response of the S-A node to cholinergic nerve stimulation applied at the S-A node was analogous to the behavior of the A-V node. The response to cholinergic stimulation in the presence of S-A nodal drive was not different from that in the presence of atrial drive. This indicates independence of the conduction block on the junction between S-A node and atrium if it is assumed that S-A nodal fibers only were involved. However, this assumption may not be valid.

In the isolated preparation, the latent automaticity of one area of specialized tissue was readily revealed when the dominant pacemaker was suppressed by nerve stimulation. Many similarities in the electrophysiological properties of the two nodal regions exist. Nevertheless, the dominant automaticity of the S-A node probably is dependent upon differences in membrane permeability to those ions associated with the rate of slow diastolic depolarization (15) and upon differences in the adrenergic innervation of these two structures.

Acknowledgment

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NERVE STIMULATION IN THE A-V NODE


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