Intrinsic Innervation of the Canine Heart

Effects on Conduction in the Atrium, Atrioventricular Node, and Proximal Bundle Branch

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SUMMARY. The cardiac neural elements which survive surgical denervation of the heart have been called the "intrinsic" innervation (ICN). These include postganglionic parasympathetic neurons and chromaffin cells. Specific activation of the ICN with nicotine (Nic) has been shown to produce powerful negative inotropic and chronotropic effects on the heart. The present study was performed to assess the influence of the ICN on dromotropic activity. Seven mongrel dogs successfully underwent surgical denervation of the heart. They were placed on cardiopulmonary bypass and conduction through the atrioventricular (AV) node as well as the right atrium was measured by an acrylic electrode plaque. Conduction through the right bundle branch (RBB) was measured using a second recording electrode. Drugs administered were acetylcholine (ACH, 0.1-10.0 µg), Nic (1-100 µg), and tetrodotoxin (TTX, 30 µg). All agents were injected by the intravenous route. Both ACH and Nic caused significant increases in AV nodal conduction time; i.e., 5-49% and 11-49%, respectively. Intra-atrial, bundle branch, and His-to-ventricular muscle conduction was affected inconsistently, and the changes were nonsignificant. Responses to Nic, but not to ACH, were completely blocked by 30 µg of TTX. The effects of the ICN on ventricular conduction appear to parallel those of the parasympathetic innervation, i.e., profound influence on AV nodal conduction with little or no effect below this structure. In contrast, however, the ICN does not appear to affect intra-atrial conduction, nor do there appear to be functionally important chromaffin cells that affect conduction time. Circ Res 47: 74-79, 1980.

Recent reports from this laboratory (Priola and Spurgeon, 1977; Priola et al., 1977) have shown that the intrinsic cardiac innervation (ICN) is capable of producing powerful negative inotropic effects on both atrial and ventricular contractility. These responses were elicited by nicotinic stimulation of parasympathetic postganglionic neurons in both intact and cardiac-denervated animals. Because these neural elements survive surgical denervation, they are referred to as the "intrinsic" nerves of the heart. The present experiments were performed to determine the extent of the effects of these intrinsic cardiac nerves on conduction within the heart. The results comprise the first demonstration of the ability of the ICN to modulate dromotropic activity of the heart.

Methods

Dogs (15.5 ± 0.5 kg) were selected without regard to sex or breed and were subjected to extrinsic surgical denervation of the heart using the two-stage technique of Geis et al. (1971). They were anesthetized with Pentothal sodium (20 mg/kg), supplemented as necessary (6 mg/kg), iv. This procedure involves separation of the heart from its mediastinal attachments in two separate procedures. The first operation involves circumferential stripping of the adventitia from the pulmonary artery and the aorta as well as separation and reanastomosis of the left atrial free wall from the inferior to superior interatrial septum. The second operation involves transection and reclusion of the interatrial septum under inflow occlusion with subsequent separation and reanastomosis of the right atrial free wall from the inferior to the superior interatrial septum. Two to 4 weeks after the second operation, the dogs were tested for the adequacy of denervation prior to their being used in the acute phase of the study. Testing included stimulation of both cervical vagi, both stellate ganglia and intracoronary injection of 500 µg of tyramine. Any dog that showed a change of 10% or more in either heart rate or AV nodal conduction was eliminated from the study. Responses of <10% were considered acceptable when compared to those of innervated animals in which either asystole or complete AV block characterized the usual responses to vagal stimulation, and changes of 100-300% were not uncommon in response to tyramine or stellate stimulation. Denervation was considered adequate in
seven animals, and the data from these animals are reported.

For the acute studies, the dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) supplemented with intravenous thiopental sodium (6 mg/kg) as necessary. Cardiopulmonary bypass then was established using standard techniques. Both ventricles were vented using sumps under negative pressure. An acrylic electrode with five recording sites (Priola et al., 1969) then was sutured over the region of the bundle of His through a right atriotomy. After closure of the atriotomy, the right ventricle was opened using a 3-cm incision parallel to the interventricular septum. The right bundle branch about 1 cm proximal to the right anterior papillary muscle was located visually and verified electrically with an exploring electrode. A high-frequency spike which followed the His bundle potential by ~25 msec but clearly preceded the depolarization of the basal interventricular septum was identified as the right bundle branch (RBB) potential. After the location of the RBB, a similar acrylic electrode was attached to the right interventricular septum directly over the area where the RBB potential was observed. Care was taken during attachment of the electrodes to place the silk sutures >2 mm away from the His bundle or right bundle branch. The sutures were placed so as not to interfere with the known blood supply to the tissues of interest. Maintenance of stable electrical recordings throughout the experiment indicated a lack of experimental trauma. Both His bundle (HB) and RBB potentials were filtered with a low cutoff of 80 Hz and a high cutoff of 1 kHz. Hearts were paced from the right atrial appendage at 120–172 beats/min (bpm) (average 147 ± 5 SEM). A lead II ECG also was recorded for timing purposes.

All potentials were amplified by Tektronix FM 122 preamplifiers and recorded by an Ampex SP 300 tape recorder at 7.5 ips. The output from the playback heads was monitored to observe the responses. Permanent records were made by playing the data back at 1-7/8 ips into a Grass polygraph at a paper speed of 100 mm/sec. This permitted a 4X time base expansion and allowed measurement of intervals to an accuracy of ±1 msec. All control intervals were measured to ensure that the interval designated as "control" was stable (i.e., ±1 msec). Intervals corresponding to the maximum changes in response to any experimental procedure were measured and compared to control to compute percent change. Again, all response intervals were surveyed to ensure that the interval selected as maximum response was not a step-function change or an aberrant cycle. Student's t-test was employed to determine significance of differences between average values. Figure 1 shows the average time course of the response of the A-H interval to the intracoronary injection of both NIC and ACh. The two curves are not significantly different. The responses reach a maximum within 2–3 seconds following injection and are complete after about 7 seconds. Usually, only about two cardiac cycles showed the maximum change. This value was measured and reported as the experimental response.

All drugs were injected via a catheter whose tip was located in the ascending aorta just distal to the aortic valve. Under bypass conditions, the injected flows directly into the coronary circulation. Acetylcholine (ACh), 0.1–10 μg, was injected to examine the dromotropic changes in response to muscarinic receptor activation. Nicotine (NIC), 1–100 μg, was injected to examine the dromotropic responses which occur in response to nicotinic activation of the intrinsic nerves (ICN). To verify that the NIC responses were mediated through intracardiac neural structures, tetrodotoxin (TTX), 30 μg, was injected by the intracoronary route to block activation of the ICN. The integrity of the effecter cell responsiveness was then tested by a second series of ACh injections.

**Results**

Representative responses of one denervated dog to ACh are shown in Figure 2. The heart is paced from the right atrium (near the sinoatrial node) at 160 bpm throughout. The A wave is small because the gain was decreased as a result of the His and ventricular potentials being quite large in this recording. In the upper panel, the control recordings show a pace to atrium (P-A) interval of 115 msec, an atrium to His bundle (A-H) interval of 53 msec and a His bundle to basal interventricular septum (H-V) interval of 91 msec (upper channel). The lower channel shows a recording taken from the region of the right bundle branch (RBB) which generates the slow, negative spike preceding the large depolarization of the ventricular septum (rifs). The control values for the His to RBB (H-
The right panels, similar responses to 5 µg of NIC are shown. Again, NIC fails to elicit any changes in the H-V, H-RBB and rb-rivs intervals. The P-A increases only slightly; i.e., 4%. However, the A-H interval increases by 100% from 55 to 110 msec (bars, lower panel). Therefore, the response of the A-H interval to NIC was dose-related. In many experiments NIC, even at low doses, slowed AV nodal conduction sufficiently to produce complete block.

Dose-response curves for the A-H interval are illustrated in Figure 4. The average responses shown are statistically indistinguishable at all doses. Although the dose of NIC was 10x the dose of ACH, the shapes of the curves are quite similar except for the one point at 2.5 µg ACH. Both the threshold and maximum responses are similar; i.e., an average of 50% increase in A-H interval could be obtained before complete AV nodal block supervened. The similarity in the curves also suggests identical cholinergic mechanisms for producing the dromotrophic change, i.e., activation of muscarinic receptors. However, the differential blockade by TTX (Fig. 5) clearly indicates that the effect of NIC requires a neuronal intermediary whereas that of ACH is direct.

The average responses as well as the control values from all seven denervated animals used in this study are summarized in Table 1. In all cases, the changes in P-A interval produced by either ACH or NIC were small and variable; i.e., no consistent effect on overall conduction in the right atrium was observed. Similarly, the H-RBB interval showed no consistent response to either muscarinic or nicotinic activation. In contrast, both ACH and NIC caused significant prolongation of AV nodal conduction as seen in the positive changes in the A-H interval at all doses studied. There is generally a positive relationship between the dose of agonist and the change in the A-H interval. The response to NIC appears to reach a plateau at 50 µg, usually because AV block supervened at doses >25 µg. Although the summed data for the other intervals are not shown, no consistent changes were produced by either ACH.

**Figure 2.** Responses to ACH, 0.5 µg (mcg). Control recordings are on top and maximum responses to the drug are at the bottom. Records are from His bundle electrode (His) and an electrode on the right bundle branch (RBB). Control (C) and experimental (E) A-H and H-RBB intervals are shown as the bars to the right of each lower panel for easier comparison of the changes produced by ACH in these intervals. P = pace artifact; A = distal right atrial depolarization; H = depolarization of His bundle; RBB = depolarization of right bundle branch; rivs = depolarization of right interventricular septum; V = depolarization of basal interventricular septum. Traces darkened for clarity. The bracket in the upper control recording indicates the A-H interval used for measurement.

RBB and RBB to right interventricular septum (rb-rivs) are 50 msec and 26 msec, respectively. Maximum responses to 0.5 µg of ACH are shown in the lower panel. The only interval which is significantly changed in response to the drug is the A-H which increases to 69 msec or 30% (bars, lower panel). The P-A is increased slightly to 122 msec (6%).

Figure 3 illustrates typical responses to two doses of NIC observed in this study. The upper left panel shows control recordings. The values for the P-A, A-H and H-V intervals are 114 msec, 56 msec, and 103 msec, respectively. The intervals in the RBB recordings measure 15 msec (H-RBB) and 50 msec (rb-rivs). As seen above with ACH, 2 µg of NIC causes significant changes only in the A-H interval. This indicator of AV nodal conduction time is increased by 38% to 60 msec (bars, lower panel). In
or NIC in the H-V or rlv-riv intervals. This suggests that neither agent has a significant effect on overall intraventricular conduction (i.e., His bundle activation to terminal activation of the basal interventricular septum).

Based on the results of earlier studies (Priola and Spurgeon, 1977; Priola et al., 1977), it was presumed that the responses to NIC were mediated via the intrinsic cardiac nerves whereas the responses to ACh were the result of activation of muscarinic receptors on the effector cells themselves. To verify this assumption, NIC and ACh were administered to all animals following intracoronary injection of 30-60 μg of TTX. If the assumption is valid, this neurotoxin should block the NIC response while leaving those to ACh unaffected. Figure 5 illustrates typical responses following TTX. The recordings are taken from the same animal as in Figure 3. After TTX, administration of 0.5 μg ACh (left panels) causes a 60% increase in the A-H interval (55-88 msec), a larger change than that observed before TTX. All other intervals remained essentially constant. In contrast, 6 μg of NIC (right panels) elicited no significant changes in any interval including the A-H. Thus, the predicted selective blocking action of TTX was observed.

**Discussion**

Previous work has shown that the intrinsic cardiac nerves (ICN) can be activated specifically by NIC and can cause profound negative inotropic and chronotropic effects (Priola et al., 1977). These studies demonstrated that the ICN were present in both the atria and the ventricles, although their

**Figure 4** Dose-response curves for the response of the A-H interval to NIC and ACh. Each point represents the mean of seven experiments and the bars represent the SEM. Ordinate is in percent change from control and abscissa is in μg of ACh and NIC (×10). For control values at each dose, see Table 1.

**Figure 5** Effects of 30 μg of TTX on test responses to ACh (left panels) and NIC (right panels). TTX was given 5-10 minutes prior to injections. Control recordings at top, experimental recordings at the bottom. Layout and abbreviations identical to Figures 2 and 3.

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**Table 1** Control Values and Responses of Denervated Dogs to Acetylcholine and Nicotine prior to Neural Blockade with Tetrodotoxin

| Acetylcholine | Nicotine |
|--------------|----------|
| Dose (μg)    | P-A | A-H | H-RBB | Dose (μg) | P-A | A-H | H-RBB |
| 5            | 0.3 ± 2.9 | 10.7 ± 8.1 | 0.0 ± 6.0 |
| (90.0 ± 13)  | (56.5 ± 5) | (22.5 ± 6) |
| 0.5          | -2.6 ± 1.8 | 5.4 ± 1.9 | -1.3 ± 1.3 |
| (74.6 ± 7)   | (56.5 ± 5) | (25.1 ± 4) |
| 1.0          | -0.4 ± 2.0 | 14.6 ± 3.3 | -1.5 ± 2.0 |
| (80.7 ± 9)   | (55.9 ± 7) | (21.8 ± 5) |
| 2.5          | -0.6 ± 2.7 | 9.0 ± 5.1 | 0.2 ± 2.1 |
| (75.1 ± 9)   | (52.6 ± 5) | (25.9 ± 7) |
| 5.0          | 10 ± 1.3 | 49.2 ± 25.7 | 11.3 ± 4.3 |
| (88.2 ± 10)  | (57.7 ± 4) | (21.3 ± 6) |

*All responses are percent change from control given as mean ± SEM. Average control values (± SEM) in milliseconds are shown in parentheses beneath each percent change value.

1 Interval from pace artifact to distal right atrial depolarization.

2 Interval from distal right atrial depolarization to depolarization of His bundle.

3 Interval from His bundle depolarization to depolarization of right bundle branch.

4 P < .05 as compared to control values (Student's t-test).
concentration in the latter was considerably less than in the atria based on the degree of negative inotropic produced. The changes produced by NIC were remarkably similar to those reported earlier for vagal stimulation in both a quantitative and qualitative sense (Priola et al., 1977; Priola and Fulton, 1969). For example, atrial effects were greater than ventricular and showed significantly greater rates of change of inotropic effects than the ventricles. NIC administration also produced abrupt bradycardia which, along with the inotropic effects, was abolished by TTX. This chronotropic response was the only observation suggesting the electrical actions of the ICN.

The present study shows that, at constant heart rate, the ICN is capable of producing negative dromotropic effects on the AV node. The maximum dose of NIC reported in this study causes an average increase in AV nodal conduction time of approximately 50%. Higher doses usually produced complete AV block characterized by a complete absence of His bundle depolarization. Occasionally, a small abnormal potential might appear in the His electrogram, suggesting failure of conduction in the upper His bundle. If the ICN is indeed the responsible neural apparatus, a significant effect on the AV node would be expected because this area is normally the recipient of dense vagal innervation (Yamauchi, 1973).

As is clear from Figure 1, the time courses of responses to Ach and NIC were essentially identical. This might be anticipated because the terminal mechanism for each response is cholinergic activation of muscarinic receptors. It is somewhat surprising, however, because the responses to NIC require as an intermediate step stimulation of the ICN and therefore might be expected to show a greater latency. The fact that the two agents are indeed acting by different mechanisms is clear from the differential blocking effect of TTX (Fig. 5). Thus, it may be that the rate-limiting step is the activation of the muscarinic receptor or the change in ionic conductance of the AV nodal cell membrane and the time required for nicotinic stimulation of the ICN is but a small part of the total response.

In intact animals, the effects of NIC include stimulation of nicotinic receptors on sympathetic postganglionic nerve endings causing release of noradrenaline (Lee and Shideman, 1959; Su and Bevan, 1970). This typically produces a biphasic response; i.e., initial depression followed by a longer-lasting excitation. In denervated animals, this latter response would not be expected because of the degeneration of the sympathetic fibers which follows extrinsic neuratomy. This was indeed the case in this series of animals; i.e., biphasic responses were not observed in response to NIC—only negative dromotropy was seen. Additionally, if functionally important chromaffin cells existed in the region of the AV node (Ellison and Hibbs, 1974; Trues, 1980), NIC might be expected to activate them and produce some positive dromotropic effects via this mechanism. The lack of appearance of any positive effects on the AV node in denervated animals also, then, precludes the existence of any functionally important chromaffin cells contributing to the influence of the intrinsic innervation on the AV node. This same conclusion was reached as a result of a recent study on the inotropic and chronotropic effects of ICN stimulation (Priola et al., 1978).

It is well known that vagal stimulation can cause an increase in atrial conduction velocity (Hoffman, 1977). The mechanism of this effect is considered to be an Ach-induced increase in gK* with a subsequent hyperpolarization and an increase in the dV/dt of phase zero depolarization (Hoffman, 1977; Bailey et al., 1972; Hutter, 1961). In the present study, neither Ach nor NIC caused any consistent changes in the P-A interval, an overall measure of conduction time through the right atrium (Table 1). It is entirely possible that the lack of significant effect was the result of an insufficient Ach concentration at the effector sites. Neuromuscular released Ach would be expected to be at a very high concentration in the immediate vicinity of the effector cell, whereas injected Ach would have to be given in massive doses to attain the same local concentration (Levy and Zieske, 1969). Thus, the lack of dromotropic effect may very well be dose-related at the receptor itself. It is also possible that activation of the ICN by NIC is an insufficient stimulus to attain maximum postganglionic Ach release.

As can be seen from Figures 2 and 3 and Table 1, the ICN has no detectable effect on the proximal ventricular specialized conduction system. Any changes observed were small and of variable direction. Clearly, conduction times from the His bundle to the mid- or distal right bundle branch could be measured accurately and show essentially no change (Table 1). These data also correlate well with the relative sparsity of innervation in the region of the common bundle and the bundle branches as compared to the AV node (Yamauchi, 1973). The lack of observed effect on the RBB and intra-atrial conduction is not the result of the surgery involved in attaching the electrodes, because the AV node was quite responsive to both Ach and NIC, even though the electrode was attached in the same way as the RBB electrode.

Overall conduction times from the His to the basal interventricular septum (H-V) or from the right bundle to the underlying interventricular septum (rb-rvs) also were unchanged by NIC (and Ach), suggesting that distal intraventricular conduction also was unaffected by nicotinic activation of the ICN. This does not mean that more subtle changes in conduction velocity or pattern in the peripheral Purkinje system could not be occurring or that coupling times from Purkinje fibers to ventricular muscle might not be affected. Also, changes in rates of repolarization, refractory periods, and action potential duration could have been produced,
but our measurements would not be able to detect these so long as they did not affect overall conduction times.

The lack of obvious effect on the bundle branch is not surprising in view of earlier work (Priola, 1973) which showed that loss of vagal tone, maximal sympathetic stimulation, and supra-blocking doses of propranolol failed to cause any significant changes in the H-RBB or H-LBB (His to left bundle branch) intervals. In short, the proximal ventricular conduction system appears strikingly nonresponsive to any kind of autonomic stimulation. Our results and those of others (Varghese et al., 1973) are somewhat difficult to reconcile with the results of experiments which show a definite vagally mediated “stabilization” of the ischemic ventricle (Hoskins et al., 1972; Kent et al., 1974) or an increase in the threshold for ventricular fibrillation (Kent et al., 1974). Certainly, in vitro studies have shown that ACh hyperpolarizes isolated Purkinje fibers which have previously been depolarized and increases the phase 0 dV/dt which should lead to an increased conduction velocity (Bailey et al., 1972). In our studies, however, the Purkinje system was probably normal, so that this effect of ACh would have been minimal. The effective ACh concentration again may be a problem; this can be much higher in vitro than in vivo because of the effects of ACh on the in situ heart (i.e., atrial fibrillation, AV block). Also, there may be a difference in responsiveness between Ringer’s-perfused and blood-perfused systems (Rosen et al., 1972). It is also conceivable that changes in intraventricular conduction are either at the limits of measurability or consist of changes in conduction pattern, homogeneity of repolarization, and refractory periods, which would have to be measured with multiple electrodes. This would make them difficult to detect individually although, in the aggregate, the effect may be functionally significant. Notwithstanding these possibilities, our experiments clearly indicate that functionally observable responses (i.e., conduction times) of the specialized cardiac conduction system to stimulation of the intrinsic cardiac nerves are limited to the AV node and, perhaps, the upper His bundle.

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