Intrinsic neural regulation of the heart in the chronic, conscious dog

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Priola, Donald V., Xiaoling Cao, Constantine Anagnostelis, and Eberhard Bassenge. Intrinsic neural regulation of the heart in the chronic, conscious dog. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H2074–H2084, 1998.—The present experiments were performed to examine the capability of the intrinsic cardiac nerves (ICN) to modify cardiac performance in the resting chronic, conscious dog. Control and cardiac-denervated dogs were instrumented for recording of left atrial (LA) and ventricular (LV) contractility, heart rate, and atrioventricular (AV) conduction time. Acetylcholine (ACh) and nicotine (Nic) were administered via an indwelling coronary artery catheter. Limited distribution from the injection site only allowed access to the LA, LV, and AV node. Both β-blockade with timolol and cardiac denervation were used to separate direct effects of ICN stimulation from indirect (e.g., reflex) effects. ACh produced the expected negative inotropic and dromotropic changes. ICN stimulation with Nic caused large decreases in LA contractility along with depression of AV conduction but only trivial effects on the LV. We concluded that the ICN has limited effects on cardiac performance in the resting animal under minimal sympathetic drive. It is likely, however, that the ICN is capable of significantly depressing cardiac function under conditions of elevated sympathetic tone as would be encountered in exercise.

intrinsic cardiac nerves; parasympathetic nervous system; nicotine; cardiac innervation; cardiac ganglia

AFTER EXTRINSIC CARDIAC denervation, such as that produced by clinical cardiac transplantation, certain intracardiac neural elements survive. These have been referred to as the intrinsic cardiac nerves (ICN). They consist essentially of at least two major elements: 1) postganglionic parasympathetic cell bodies with their axons and 2) chromaffin cells. Both are concentrated primarily in the atria (7). The effects of ICN stimulation have been shown in the sinus node (26), atrioventricular (AV) node (22), and atrial and ventricular myocardium (7, 25, 26). The inotropic effects of ICN stimulation have also been demonstrated in vitro (6, 28, 29). Although there is little question that the ICN can influence a wide variety of cardiac functions, there is currently no evidence that this system is capable of modulating cardiac function in the normal, conscious animal. All of the previously cited work has been done either on acute, anesthetized, and surgically traumatized animals or on cardiac tissues in vitro. The purpose of the present experiments was to determine whether ICN stimulation has any significant effects on cardiac function in the resting chronic, conscious animal. In addition, by using the extrinsically denervated heart as a model of the transplanted heart, we hoped that this preparation might provide realistic information on the responses of these latter hearts to self-administered or environmental substances (e.g., nicotine) that are capable of activating the ICN.

METHODS

Selection and Conditioning

Animals were selected from the local animal shelter without attention to sex or breed. They were chosen, however, for their ability to be socialized and to be gentle when handled. All animals underwent extensive conditioning for 3–6 wk after they arrived at the Animal Resource Facility. This process ensured 1) adequate quarantine to ensure freedom from preexisting disease, 2) administration of necessary immunizations, and 3) establishment of optimal nutrition.

Surgical Preparation: Control Animals

Anesthesia was induced in these animals (n = 15) with Pentothal Sodium (20 mg/kg iv), after which it was maintained by isoflurane mixed with oxygen. The heart was approached via a left lateral thoracotomy in the fourth interspace; the ribs were spread widely. After we suspended the heart in a pericardial cradle, the left atrial appendage was retracted and the proximal left circumflex coronary artery (LCCA) was dissected free of surrounding tissue for a distance of approximately 1.5–2.0 cm. To perform chronic intracoronary injections, we inserted an indwelling coronary artery catheter into the vessel using the Herd and Barger technique (13) as modified by Bassenge et al. (4) and, later, by Gwirtz (11). Briefly, a 5–0 vascular suture with a small, curved needle at one end was tied to the tip of a small-diameter Silastic catheter (0.6-mm OD). The small catheter was ~4 cm in length and was sealed to a larger Silastic catheter (2.2-mm OD) that was long enough to be exteriorized after closure. A fine Dacron patch (Cooley Graft Knit) was attached at the point where the catheters were joined, in order to facilitate stabilization and fixation of the intracoronary catheter after insertion. The suture needle was passed in and out of the vessel, and the suture was used to pull the intracoronary catheter into and out of the vessel via the same path. After we sutured the Dacron patch firmly in place to stabilize the catheter, the distal end of the catheter was stretched ~25% and cut, and the tip was allowed to retract within the vessel. This technique usually left about 3–4 mm of the catheter tip within the coronary branch. The catheter was then checked for patency. In our experience, as with the experience of others (11), these catheters routinely stayed patent to drug injection for 90 days or more with only a daily saline or saline-heparin flush. The occasional clot could be easily cleared by filling the catheter with streptokinase for 30 min and then flushing. In addition, some animals were fitted with a second, larger pulmonary artery catheter. A shortened
20-gauge butterfly-needle catheter was inserted into the lumen of the main pulmonary artery, sealed with a purse-string suture, and fixed firmly to the adventitia with silk sutures. This catheter was used for any necessary systemic drug injections in these dogs.

Recording pacing electrodes were sutured to the right atrial (RA) appendage and to the apex of the left ventricle (LV). The electrodes consisted of a pair of pure silver balls (2.0 mm in diameter) bonded to the surface of a Dacron patch. Stranded, 29-gauge insulated stainless steel wire was attached to each silver pickup and led to the outside via a silicone-filled Silastic catheter (2-mm OD).

A pair of epoxy-embedded piezoelectric crystals (3-mm diameter) were then sutured to either side of the LV base for recording of absolute changes in LV basal diameter (LV ΔL). Similarly, a pair of smaller crystals (1 × 1 mm) was attached to the lateral wall of the left atrium (LA) oriented so that they were perpendicular to the wall and faced each other 1 cm apart. Signals from the sonometric crystals were brought to the outside using stranded, Teflon-insulated stainless steel wire (34 gauge) called and embedded in a silicone-filled Silastic catheter (2-mm OD). This technique provided recordings of absolute changes in LA segment length (LA ΔL), which, although of small amplitude, were nonetheless easily measured and gave a reasonable approximation of changes in LA mechanical activity in response to the drugs used. Typical control recordings from an innervated animal are shown in Fig. 1, along with magnified recordings of LA ΔL (top inset) and LV ΔL (bottom inset). Arrows in each inset indicate onset of systolic shortening in each recording.

After implantation was completed, the incision was closed, and all leads and catheters were stress-relieved and exteriorized between the scapulas. The animal was placed in a mesh jacket with a pocket to protect the leads and catheters and to keep them clean. The dogs were treated postoperatively with analgesics and antibiotics in a veterinary surgical intensive care unit. Recovery from the preparative surgery was usually uneventful. Combined intra- and postoperative mortality was 15%. The animals were allowed to recover for 2 wk before they were placed in the sling for the first time and data were recorded.

Surgical Preparation: Denervated Animals

These animals (n = 12) were subjected to the one-stage intrapericardial denervation procedure developed by Randall et al. (27) before implantation of the recording devices. This procedure involves separation of the atria from their mediastinal attachments, stripping the superior vena cava and the main, left, and right pulmonary arteries of their adventitia, sectioning of the azygous vein, and stripping of the left superior pulmonary vein with section of the overlying ventrolateral cervical cardiac nerve (VLCCN). After completion of the denervation procedure, the coronary catheter and recording devices were implanted as in the control animals. The success rate for cardiac denervation with this procedure has been virtually 100% in our hands. Denervation was verified with a number of techniques: 1) lack of any significant response to intracoronary (ic) injection of 50-500 μg of tyramine; 2) lack of chronotropic, dromotropic, or inotropic response to 2 μg ic of veratridine; 3) absence of respiratory sinus arrhythmia; 4) absence of beat-to-beat variability in the interval between atrial systole (ALa) and ventricular systole (VLa) as measured by depolarization of the respective chambers (see Data Recording); 5) absence of a secondary positive response to intracoronary injection of nicotine (Nic), and 6) increased responsiveness to intracoronary injection of 0.025 μg norepinephrine (NE), i.e., denervation supersensitivity.

Data Recording

The piezoelectric atrial and ventricular crystals were activated and detected by a two-channel ultrasonic system (Tri-ton Technology model 120). Both channels were calibrated at each session, and triggering was adjusted for maximum stability. The outputs were directed to the inputs of Gould universal amplifiers. The outputs of these amplifiers were directed into differentiator circuits so that the rate of change of each length (LA dL/dt and LV dL/dt) could be directly recorded. RA and LV electrograms were amplified by Gould universal amplifiers filtered to record in the range 30 Hz–1 kHz. In addition to recording each electrogram directly, the RA electrogram was directed to the input of an interval cardiotachometer (Gould Biotech module) for beat-to-beat recording of heart rate (HR). Both RA and LV electrograms were directed to the input of a specially designed circuit (H. Weise, Univ. of Freiburg) that measured the interval between the two potentials on a beat-to-beat basis (Aa-V), providing a good estimate of instantaneous changes in AV conduction time. All eight recordings were then directed to an analog-to-digital converter (TL-2–40 Labmaster DMA, Axon Instruments), which digitized the recordings so that they could be acquired by personal computer-based data display, storage, and analysis software (AxoTape40, v1.2.02, Axon Instruments). All data were stored on a hard disk and transferred later to floppy diskettes for analysis and permanent storage.

Experimental Protocols

The first two or three times the animals were placed in the sling, the leads were connected, the channels were calibrated, and the animals were released from the sling in 15–30 min. Although the data from these sessions were recorded for archival purposes, they were not used for experimental values. After the introduction to the laboratory environment in this way, all animals became quite comfortable and were usually content and quiet for data recording periods of up to 1–1.5 h. In the innervated animals (Fig. 1), the lack of stress was evidenced by respiratory sinus arrhythmia, HR of <100 beats/min (average: 95 ± 7 beats/min), and relaxed behavior (i.e., most dogs slept, off and on, during recording sessions). Similarly, calm behavior was the rule in the cardiac-denervated animals (Fig. 2), but their HR were consistently >100 beats/min (average: 103 ± 1.1 beats/min). Neither the HR nor the Aa-V recording displayed any discernible respiratory variations. All intracoronary injections were done via the indwelling coronary catheter and, when necessary, systemic injection was done using either the pulmonary catheter or an Intracath inserted into a cephalic vein before the animal was placed in the sling. Aseptic procedures were observed so that catheter or lead contamination was rarely observed. After the recording period, animals were rewarded with dog biscuits and play time before being returned to the Animal Resource Facility. The guiding principles for humane treatment of experimental animals of the American Physiological Society were scrupulously observed.

All tested drugs were routinely injected intracoronarily in order to restrict drug actions to the heart. In addition, the injection site in the proximal LCCA only permitted drug to reach the LA, LV, AV node, and, to a lesser degree, the right ventricle (RV). Little or no drug reaches the SA node from this site (see DISCUSSION). Consequently, any observed effects on sinus rate were the result of indirect (i.e., reflex) activation of the SA nodal innervation. Nic, in doses of 2–100 μg, was used to stimulate the cell bodies of the ICN, which then initiated axonal release of ACh, causing typical muscarinic cardiac...
effects. ACh itself, in doses of 0.1–5.0 µg, was used to directly activate cardiac muscarinic receptors. When necessary to eliminate concurrent adrenergic effects, Nic-stimulated catecholamine (CA) release was blocked by 2 mg timolol ic. Other drugs used were tyramine (to test for the presence of neurally stored CA), NE (to test the adrenergic sensitivity of the cardiac effector cells; see Surgical Preparation: Denervated Animals), and veratradine (to test for the intactness of afferent and efferent vagal fibers that mediate veratradine-induced bradycardia).

Because of the variability in control levels, even in the same animal, all results were normalized by expressing them as percent change from the immediate, preinjection control value. The statistical significance of any difference in values was evaluated either by a paired t-test or, when appropriate, by ANOVA.

RESULTS

Responses to ACh

Atrial contractility. The responses of the LA of the control animals to ACh are summarized in Fig. 3A. At all doses of ACh, the LA showed decreases in both the extent and rate of shortening (only the changes in LA ΔL are shown). This is consistent with a direct action of
ACh on muscarinic receptors in atrial muscle. It should be noted that no clear dose-response relationship is apparent. This is typical for cholinergic effects on contractility that we have reported previously for the acute, isovolumic canine heart preparation (25, 26, 29). As in these previous studies, decreases in atrial contractility were in the range of −30 to −60%. In many instances, atrial shortening became zero for a number of cycles, even though atrial electrical activity was uninterrupted. These responses were essentially unaffected by prior β-blockade with timolol. The responses of the cardiac-denervated dogs to ACh are summarized in Fig. 3B. As in Fig. 3A, the inotropic data were compared with the same responses in the control animals after β-blockade. Unlike the control animals, the LA of cardiac-denervated animals showed a recog-
nizable dose-response relationship. In addition, the LA of the cardiac-denervated animals was more sensitive to ACh than that of the control dogs; i.e., two of the cardiac-denervated animals showed clear responses to ACh at a dose of 0.025 µg (−16 and −60%), whereas none of the control animals was ever found to respond at an ACh dose lower than 0.10 µg. However, except for the 0.5-µg dose, responses of the two groups are not significantly different when compared over the same dose range.

Ventricular contractility. In contrast to the LA, LV mechanical responses to ACh in the control animals were small to nonexistent (Fig. 4A). This contrasts sharply with previous studies using the canine isovolumic heart preparation (25, 26), in which consistent negative inotropic responses of 10–30% were observed. Ventricular inotropic responses were also unaffected by β-blockade. The LV of the cardiac-denervated animals showed responses to ACh that were essentially identical to those of the control group (Fig. 4B). Again, there was no discernible dose-response relationship observed throughout the dose range studied. The response to 5.0 µg ACh appears to be negative, although the change was relatively small (10–15%).

HR. In sharp contrast to the mechanical responses, chronotropic responses to ACh were uniformly positive, ranging between 0 and 20% (Fig. 5A). In addition, there appeared to be a shallow, but demonstrable, dose-response relationship. After β-blockade, the dose-response curve was shifted to the right, suggesting that there had been an adrenergic component in the chronotropic responses observed before blockade. However, the degree of tachycardia observed at 5 µg ACh was unchanged by β-blockade. In contrast to the control animals, there was no significant change in HR in the
cardiac-denervated group at any dose of ACh studied (Fig. 5A).

AV conduction (A-V₅). In the control group (Fig. 5B), intracoronary ACh uniformly increased AV conduction time. Also, there was a clear dose-response relationship in the range of 40–120%. In fact, at the higher doses (5 and 10 µg) complete AV block was a frequent observation. It should be noted that this contributed greatly to the average values because complete AV block was arbitrarily recorded as a percent change of 150. In contrast to the chronotropic data, β-blockade did not significantly alter AV nodal depression by ACh, suggesting the absence of any significant adrenergic contribution to the control responses. When the ACh responses of the AV node in the control group are compared with those of the cardiac-denervated animals (Fig. 5B), the dose-response curve appears to be shifted to the right, indicating a decreased sensitivity to ACh.

Responses to Nic

Atrial contractility. Intracoronary injection of Nic in conscious control dogs uniformly resulted in strong negative inotropy in the LA (Fig. 6A), ranging between −30 and −80%. Before β-blockade these responses did not exhibit a clear dose-response relationship. After blockade the decreases in contractility appeared to be dose related over the range of 1–50 µg Nic. After surgical denervation of the heart, all cardiac neural connections to the central nervous system are interrupted, including cardiac afferents, all sympathetic nerves, and all vagal preganglionic nerves (24, 27). What remains are vagal postganglionic neurons and CA-containing chromaffin or small intensely fluores-

cent cells, the elements that comprise the ICN. Consequently, those responses that were influenced by the effect of Nic on different structures (e.g., chemoreceptors) (10) or by its effect on NE release from adrenergic neurons (32) will be altered and should now only reflect the stimulation of the ICN by Nic. This is clearly shown in the inotropic responses of the LA in chronic, cardia
denervated animals (Fig. 6B). The negative inotropic responses, although somewhat smaller because of the absence of adrenergic inhibition (see Discussion), are clearly related to the Nic dose, reaching a maximum response of about −50%.

Ventricular contractility. In contrast to the effects observed in the atria, Nic administered to control animals before β-blockade produced positive inotropic responses in the LV (Fig. 7A). The changes produced in LV segment length (i.e., ΔL) were relatively small. However, data derived from a different index of contractility (i.e., dL/db) show positive inotropic responses that were more impressive (Fig. 7, inset). This index was increased 5–75% and also demonstrated a clear relationship to the dose of Nic over the 1- to 50-µg range. After β-blockade, the positive responses were eliminated and only small, negative responses were evident at the lower three doses. Clearly, the positive inotropic responses to Nic in the control animals were adrenergically mediated. Ventricular responses to Nic were small to nonexistent in the cardiac-denervated animals (Fig. 7B). This not only reflects the loss of intramyocardial adrenergic nerve fibers but also the relative sparseness of ventricular parasympathetic innervation (9, 33).

HR and A-V₅ interval. Before adrenergic blockade, control animals responded to all doses of Nic with a moderate, non-dose-related bradycardia (Fig. 8A). Paradoxically, the bradycardia appeared less prominent at the two highest doses. The effects of Nic on AV nodal conduction (i.e., A-V₅ interval) were also minimal (Fig. 8B). At the five lower doses they ranged from 0 to 10%. However, moderate decreases were evident at the two highest doses. After β-blockade, the character of the chronotropic response changed markedly; i.e., there was a definite dose-related bradycardia over the entire range of Nic injections (Fig. 8A). In contrast, AV nodal responses after adrenergic block (Fig. 8B) were relatively small and quite variable and bore no clear relationship to the dose of Nic employed. Because of the confusing and seemingly contradictory nature of these responses, the use of surgically denervated chronic animals was essential in order to isolate the role of the ICN in cardiac responses to Nic.

Clear examples of the unmasked effects of pure ICN activation are seen in the effects on both HR and AV conduction (Fig. 8). The HR, which had shown a clear dose-related bradycardia in the control animals, now is completely abolished. Because we would not expect the intracoronary Nic to reach the SA node in this preparation, the bradycardia seen in the control group probably represented a reflex vagal response to LV chemoreceptor stimulation by Nic, the so-called “Bezold-J arische” reflex (5, 17) (see Discussion). Finally, AV conduction, which demonstrated an erratic response pattern in the control animals, now clearly shows a dose-related nega-

**Fig. 6.** Dose-response curves showing effects of nicotine (Nic) on LA ΔL in control (A; ●) and cardiac-denervated conscious dogs (B; □). In A and B, responses of control animals after β-blockade (○) are shown for comparison. Ordinates show % change in LA ΔL from control levels, and abscissas show ic dose of Nic in µg on a logarithmic scale.
dromotropic effect, consistent with a direct action of Nic on AV nodal ICN. As noted in DISCUSSION, the dromotropic responses in the control animals were unpredictable because of the competing influences of NE release, direct actions on vagal neurons, and the enhancement of AV conduction secondary to bradycardia. The current protocol did not permit us to detect any effects of Nic on intracardiac chromaffin cells. However, in some preliminary experiments on atropinized cardiac-denervated animals, we have seen some minor effects on HR and AV conduction that could be attributed to these intrinsic neural elements (29).

**DISCUSSION**

**Interpretation of Data**

Although the use of a conscious animal model has the advantage of more closely reproducing the normal functioning of physiological systems, it has the disadvantage of allowing less control over the parameters of interest. In our earlier work in the anesthetized animal on cardiopulmonary bypass, drugs could be administered into both coronary arteries simultaneously so that their effects on all facets of cardiac function could be evaluated concurrently. However, in the current model, only a limited area of the heart can be accessed from the injection site. Therefore, the interplay of multiple factors must be considered when attempting to interpret the physiological data. The most critical of these are 1) the limited distribution of drugs injected into the indwelling coronary catheter; 2) reflex changes in inotropy, chronotropy, or dromotropy in areas outside of the drug’s distribution, caused by the effects of the drug within its area of distribution; and 3) multiple and/or conflicting actions of the injected drug on afferents (e.g., chemoreceptors), CA release from postganglionic adrenergic nerves, stimulation of intracardiac neurons (ICN), or direct actions on cholinergic receptors. However, we were able to dissect, with some precision, the individual components of the cholinergic and nicotinic responses by examining them before and after adrenergic blockade and by comparing their effects in normal and cardiac-denervated dogs.

Drugs injected into the indwelling coronary catheter would be expected to reach the LA, LV, and AV node directly. It is unlikely that any significant amount of injectate reaches the RA or the SA node. In previous studies where this issue was approached directly, Gwirtz (11) and Gwirtz and Stone (12) carefully documented the fact that drugs injected via this catheter did not have direct access to the SA node. This conclusion is also supported by our data, if one compares the HR responses of the normal, post-β-blockade (control), and cardiac-denervated animals to Nic as shown in Fig. 8A. The control animals demonstrate a clear, dose-related bradycardia in response to increasing doses of Nic. However, the cardiac-denervated animals display a complete lack of responsiveness to these same doses of the drug. Clearly, the bradycardia observed in the control group required intact cardiac innervation and most likely represented the chronotropic component of the Bezold-Jarisch reflex initiated by nicotinic stimulation of LV chemoreceptors (10). The complete absence of response to Nic in the cardiac-denervated animals indicates that the drug did not have access to the SA node in our preparation.
On the other hand, the conflicting nature of some of the data is shown nicely in the LV responses of the normal animals to NIC before and after β-blockade (Fig. 7A). Before adrenergic blockade, there is a significant increase in LV contractility that appears to be dose related. However, after β-blockade, this response is reversed or eliminated. We interpret these data to indicate that NIC releases CA from ventricular adrenergic nerve endings and also simultaneously activates the ICN, releasing ACh. The net result of these competing neurotransmitters is an increased inotropy because of the dominance of adrenergic fibers over cholinergic elements in the ventricular myocardium. After blockade, only the weak negative inotropic effect of activating a sparse population of ventricular ICN is observed. This interpretation is supported by the data of Fig. 7B, which show that, in the cardiac-denervated animal without β-blockade, injection of NIC has insignificant effects on LV contractility. This lack of a positive inotropic response to NIC would be expected if the postganglionic adrenergic fibers from which CA are released by NIC are no longer present in the extrinsically denervated heart. The latter appears to be the most reasonable interpretation of the data.

Direct Effects of Cholinergic Stimulation

ACh clearly decreased LA contractility (Fig. 3A), presumably by a direct action on atrial muscarinic cholinergic receptors. Responses of the chronic, conscious dog were very similar to those observed earlier in controlled, acute preparations. In the latter, intracoronary injection of 1 µg of ACh routinely produced changes of −50 to −60% in atrial contractility (25, 29). In the current experiments, this same dose caused a 30–40% decrease in LA contractility (Fig. 3A). Except for the 0.5-µg dose of ACh, β-blockade did not significantly alter LA responsiveness to ACh. This is understandable because, in the preparation at the doses employed, ACh has little if any nicotinic action on adrenergic nerve fibers and thus does not produce CA release. The LA of the cardiac-denervated animals responded to ACh with a pattern quite similar to that of the control animals (Fig. 3B). If one compares the responses of the cardiac-denervated group to that of the control animals after β-blockade, there appears to be no evidence of denervation supersensitivity. We have previously demonstrated this lack of change in sensitivity to ACh after denervation (25). The data of Fig. 3 confirm this earlier observation in the conscious, chronic animal.

The direct effects of ACh on LV inotropy were unimpressive. Both before and after β-blockade (Fig. 4A), inotropic responses in control animals varied between +5% and −5%, well within the range of spontaneous variability. This was surprising because ventricular inotropic responses to ACh in acute experiments were considerably greater (25). In these controlled, isovolumic heart experiments, 0.5 µg ACh induced decreases in LV contractility of 35%. One possible explanation for the smaller responses of the chronic, conscious animal is that, in the acute experiments, the drug reached the entire LV. In the present experiments, distribution of the drug from the site of injection most likely did not include a sufficient mass of the LV to produce large decreases in overall contractility. In addition, it is well known that muscarinically mediated changes in LV inotropy are much greater in the presence of high adrenergic activity (18, 20). The animals used in the present experiments were well acclimated to the experimental setup, usually falling asleep in the sling during much of the experiment. Consequently, their vagal tone was relatively high, whereas their sympathetic tone was undoubtedly low. The latter argument is supported by the resting HR in the intact animals, which averaged 89 ± 7 beats/min. After β-blockade, the average resting HR increased to 105 ± 8 beats/min. Because the drugs had no access to the SA node in this preparation, the increase in basal HR was probably related to β-blockade of LA and LV contractility, causing a fall in blood pressure and a reflex tachycardia. We have no alternative explanations for this increased baseline HR after β-blockade. In the cardiac-denervated animals, basal HR averaged 104 ± 5.5 beats/min. Obviously, if this was the HR in the absence of extrinsic neural influences, vagal tone must have been predominant in our control animals, with sympathetic activity at a minimum. It is possible, indeed likely, that activation of the ICN would cause a greater decrease in LV contractility if ongoing cardiac sympathetic activity were high, e.g., in exercise. Levy (19) has shown very clearly that this latter competition is important in determining the intensity of vagally mediated negative inotropic effects. Ventricular responses to ACh in the cardiac-denervated animals were also unremarkable (Fig. 4B). At the lower doses, the responses again were within the range of baseline variability. At the higher doses there was a tendency for contractility to decrease. The low sensitivity to ACh was understandable given the lack of concurrent cardiac sympathetic tone and the likelihood that plasma CA levels were also low. Again, it has been well established that ACh has a relatively small effect on ventricular contractility unless adrenergic stimulation is also present (31).

In a series of classic experiments, James and Nadeau (15, 16) studied the responses of the SA node to administration of autonomic drugs via the SA nodal artery. In this preparation, ACh produced an abrupt slowing of the nodal discharge rate, which was abolished by subsequent injection of atropine (16). In the present study, injection of ACh in the intact control animals caused a dose-related, moderate tachycardia (Fig. 5A). As discussed above, it appears that the ACh-induced tachycardia in the control animals was a reflex effect triggered by the decreases in LA and LV contractility, causing a decrease in cardiac output and hypotension. This was clearly not a direct effect of the drug on the SA node because, in the cardiac-denervated animals, the same doses of ACh cause no change in HR whatsoever (Fig. 5A). If the SA node was within the tissue domain perfused from the injection site, we would have expected a sharp, intense bradycardia. Indeed, in previous acute experiments in which agents
were injected into the aortic root on cardiopulmonary bypass, the usual chronotropic response was clearly negative (26). Thus our model of the chronic, conscious animal is unsuitable for studying direct effects on the sinus node.

Over the dose range of ACh studied, there was an almost linear increase in the A-V interval (Fig. 5B). Blockade of cardiac β-receptors did little to alter this response. However, although the SEs are fairly large, there is some decrease in responsiveness after β-blockade. These results could be explained by considering two likely components of the response. First, there is a direct inhibitory effect of the ACh on AV nodal conduction. This is a “classic” response. There is also, however, the effect of a concurrent tachycardia. As HR increases, the shortened recovery time for the node slows conduction even more. After β-blockade, the direct effect persists but the smaller tachycardia reduces the secondary effect of HR on AV conduction.

This interpretation is bolstered by the changes in A-V interval seen in the cardiac-denervated animals. Without the confounding effect of HR changes on AV nodal conduction, the direct actions of ACh on the AV node are clearer. There is a dose-related negative dromotropic effect on the AV node, but it is shifted to the right of the β-blockade curve. Again, as we have shown in other studies (25), there was no apparent denervation supersensitivity of the AV node to ACh after cardiac denervation. This lesser response might very well be the result of a lack of tachycardia in response to ACh in the cardiac-denervated animals (Fig. 5A).

Effects of Intracoronary Nic

Responses to Nic in this animal model are complex. As described above, they represent the resultant of at least three independent and simultaneous effects of the drug: 1) stimulation of the ICN (22, 29), 2) CA release (8), and 3) reflex effects of LV chemoreceptor stimulation (10). Nadeau and James (21) injected Nic directly into the SA nodal artery and also found its effects to be complex. They reported both cardiac slowing and acceleration after Nic injection; the former was blocked by atropine or hexamethonium and the latter by propranolol. They attributed these effects to ICN stimulation and CA release, respectively. The other issue that must be considered is that of “accentuated antagonism” (18).

In the atria, where the numbers of ICN are greatest, the effects of CA release may be difficult to see because of overwhelming competition from the activated ICN (e.g., Fig. 6A). In the LV, where the ICN are relatively sparse, the effects of CA release are only minimally opposed by cholinergic activity so that the effects of β-blockade are more obvious (e.g., Fig. 7A). If these factors are considered, interpretation of the effects of Nic, a common self-administered toxin in smokers, becomes clearer.

Contractility of the LA was consistently reduced by Nic (Fig. 6A). Before β-blockade, responses ranged from −30 to −60% with no clear dose-response relationship. Although the β-antagonist did not alter the overall magnitude of the Nic responses, they were more closely dose related. It seems likely that unpredictable competition between the positive influences of CA release and the negative effects of ICN stimulation would produce highly variable responses before adrenergic block. After blockade, the negative inotropic effects of Nic would be unopposed, tightening the dose-effect relationship. The magnitude of the negative atrial inotropy induced by Nic in our preparation was 2–4 times greater than that observed in our earlier acute, highly controlled experiments (25, 26). We did not attempt to determine the basis for this differential responsiveness between the two preparations although we feel that it is probably related to at least two differences between the protocols. First, the use of an anesthetic in the acute experiments may have depressed ICN sensitivity. In some preliminary experiments, we found that a very small dose of pentothal sodium (2–5 mg/kg iv) given to our conscious animals was sufficient to significantly diminish ICN sensitivity to nicotinic stimulation (unpublished data). Second, reflex connections in our control animals were intact. Activation of LV chemoreceptors by Nic triggered the Bezold-Jarisch reflex (10), which increases efferent vagal activity to the LA, as well as to the SA node (Fig. 8A). This reflex vagal activation would be additive with the pharmacological stimulation of the ICN, and the depression of LA contractility would thus be enhanced. In the majority of the acute experiments, the hearts retained their autonomic nerves, but both vagi and stellates were decentralized, obviating cardiac reflex effects on the responses.

This interpretation of the data is supported by comparing the Nic responses of the LA in the cardiac-denervated and control animals. The LA responsiveness in the cardiac-denervated animals was less than that of the control group after β-blockade (Fig. 6B) even though cardiac denervation has been shown to increase ICN nicotinic sensitivity (23, 25, 28, 29). However, the cardiac-denervated animals were not subject to the same reflex vagal stimulation produced by the action of Nic on LV chemoreceptors. The existence of this mechanism in the control group would enhance the effects of ICN stimulation and probably explains the apparent decrease in nicotinic sensitivity observed in the cardiac-denervated animals. Reversible cold block of the cervical vagus would provide a more appropriate control for Nic responses in the intact animal.

The responses of the LV to Nic are somewhat easier to understand. Before adrenergic blockade, Nic produced small, but consistent, increases in contractility (Fig. 7A). This was most apparent in the measurement of LV dL/dt (Fig. 7, inset), where the responses followed a clear dose-response relationship. These changes were related to the CA-releasing effect of Nic because they were abolished after β-blockade. The scant number of ICN in the ventricular myocardium is revealed by the small to absent changes in inotropy over the dose range of Nic studied after adrenergic blockade. This is consistent with histological studies, which have routinely failed to show significant numbers of ganglion cells in the ventricular myocardium of most species studied (9, 33). This is not to say that the ventricles are unrespon-
sive to ICN stimulation. In previous experiments, we have demonstrated changes in ventricular inotropy of −20 to −40% in response to the same intracoronary doses of Nic (25, 26). One of the reasons for this large difference in responsiveness may be related to the low level of sympathetic neural tone in our conscious animals (see Direct Effects of Cholinergic Stimulation). When sympathetic tone is low, any cholinergic response is less effective because of a lack of competition with ongoing positive adrenergic effects (30). The levels of circulating epinephrine were undoubtedly elevated in the acute preparations because of anesthesia and surgical trauma. It would be expected that a response, even from a sparse number of ventricular ICN, would produce a greater decrease in inotropy under these conditions. Another consideration is the location of the ICN cell bodies that are responsible for the negative inotropic ventricular responses. In a 1987 study, Blomquist et al. (7) clearly showed that the ICN primarily responsible for ventricular inotropic responses to Nic were located in the atria. Apparently, ICN axons are much longer than previously believed and cross the AV groove to innervate ventricular muscle. In these experiments, intracoronary Nic stimulated atrial ICN, resulting in changes of −10 to −20% in LV isovolumic pulse pressure. After chronic denervation of the AV groove with phenol, ventricular inotropic responses to Nic were abolished, whereas atrial negative inotropic responses were unchanged. In the present experiments, the LA receives blood from the injection site, but the rich population of ICN in the RA remains unaffected. This might explain, in part, the lesser effects on LV contractility in our chronic preparation compared with the acute preparation. In the cardiac-denervated group, the LV (Fig. 7B) is unresponsive to Nic both because of the paucity of ICN in the ventricles and the absence of ongoing adrenergic stimulation. The interpretation of HR responses to Nic in the control animals is simpler because the drug does not reach the SA node from the injection site (see Interpretation of Data). When the changes in LA and LV performance resulting from CA release are eliminated, a precise and predictable bradycardia is revealed, directly related to the dose of Nic (Fig. 8A). This response characterizes the chronotropic component of the cardio-cardiac reflex triggered by LV chemoreceptor stimulation, the classic Bezold-Jarisch phenomenon (3, 10).

The responses of the AV node to Nic in the control animals are more complex of all. Here, the interplay of all the previously considered mechanisms confounds interpretation when extrinsic innervation is intact. The A$_2$-V$_s$ interval was simultaneously modulated by direct ICN activation, CA release, reflex vagal discharge, and changes in HR, i.e., increased AV nodal refractoriness. The resultant of all these interacting factors for any given dose of Nic is highly unpredictable. However, responses of HR and A$_2$-V$_s$ interval to intracoronary Nic are markedly clarified in the absence of extrinsic nerves (Fig. 8). As discussed earlier, Nic has no effect on HR in the cardiac-denervated animals because it has no access to the RA from the injection site (Fig. 8A). On the other hand, the direct effects of ICN stimulation are clearly inhibitory to AV conduction (Fig. 8B) as we have shown previously in acute experiments (22). The AV node displays a classic dose-response curve, as well as the expected ICN nicotinic supersensitivity (23). Without the opposing effects of bradycardia, Nic caused a maximum average increase in the A$_2$-V$_s$ interval of ~90% at 100 µg. Whereas transient AV block was rarely observed in the control animals, the cardiac-denervated group displayed AV block in 10 of 26 experiments at the 100-µg dose. In fact, the AV node was consistently the most sensitive area of the heart to stimulation of the ICN. This is not surprising because many histological studies have demonstrated that the regions of the SA and AV nodes exhibit dense populations of ganglion cells, whereas they tend to be less abundant in the atrial myocardium and very sparse, if present at all, in the ventricular myocardium.

In conclusion, these studies, although limited in scope because the nature of the coronary catheter placement, show that the ICN are fully capable of significantly modifying cardiac inotropic and chronotropic function in the conscious, resting animal. On the basis of the many studies of sympathetic-parasympathetic interaction by Levy and his co-workers (18–20), activation of the ICN ought to have more potent actions on the heart when sympathetic neural drive is elevated, for example, during exercise. ICN stimulation might become a serious problem, for example, in cardiac transplant patients exposed to either active or passive smoking, especially under conditions of physical exertion.

The potential for the ICN to modify cardiac function might be even greater than demonstrated by our studies. There are suggestions in the literature that the ICN may contain a greater variety of neural elements than those addressed in our experiments. Armour and co-workers (1, 2, 14), among others, have speculated that the ICN may include not only postganglionic parasympathetic neurons but also "local circuit" neurons, local afferent nerves as well as a wide variety of neural elements that respond to other neurotransmitters and to peptides. Although direct anatomic and/or functional evidence for these components of the ICN is still lacking, it is exciting to speculate how much more complex the issue of intrinsic neural control of the heart might be if they are shown to exist.

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