Effects of Acetylcholine on Action Potential Characteristics of Atrial and Ventricular Myocardium after Bilateral Cervical Vagotomy in the Cat

Richard J. Kovacs and John C. Bailey

SUMMARY. Acetylcholine, the parasympathetic neurotransmitter, shortens the action potential duration of cat atrial muscle cells, but not ventricular muscle cells. In mammalian species, atrial tissue receives a richer cholinergic nerve supply than ventricular tissue. To determine whether chronic withdrawal of cholinergic tone might influence the subsequent response of these tissues to cholinergic stimulation, we examined the effect of acetylcholine on the action potentials of atrial and ventricular myocytes from cats with intact vagi and cats after chronic bilateral cervical vagotomy. Following bilateral cervical vagotomy, physostigmine (10^{-6} M) failed to alter atrial tension development or action potential duration. Acetylcholine produced shortening of the action potential duration in atrial muscle from cats with intact vagi and in cats following bilateral cervical vagotomy. However, the degree of shortening produced by acetylcholine after bilateral cervical vagotomy was significantly greater (P < 0.001). In ventricular muscle from cats with intact vagi, acetylcholine did not alter action potential duration. In ventricular muscle from cats after bilateral cervical vagotomy, acetylcholine shortened the action potential duration. Maximal effect was seen at a concentration of 10^{-5} M where acetylcholine shortened action potential duration at 90% repolarization from a control value of 179 ± 4 to 150 ± 7 msec. Atropine (10^{-6} M) reversed the effects of acetylcholine. Addition of propranolol (10^{-6} M) to the superfusate or pretreatment of the animals with reserpine (2 mg/kg, ip) 24 hours before sacrifice failed to alter the response of ventricular muscle cells to acetylcholine. We conclude that after bilateral cervical vagotomy, acetylcholine produces an exaggerated effect on atrial action potential duration, and produces a direct effect on the action potential duration of ventricular myocytes. These effects are mediated through muscarinic cholinergic receptors. (Circ Res 56: 613–620, 1985)

THE effects of muscarinic cholinergic stimulation on transmembrane action potential characteristics in the mammalian heart are different in atrial and ventricular myocardium. Early in vitro microelectrode studies proved exogenous acetylcholine capable of producing direct effects on the action potential characteristics of atrial myocytes, but not ventricular myocytes, in several mammalian species (Hoffman and Cranefield, 1960). Histological studies available at that time failed to identify significant parasympathetic input to the ventricle (Nonidez, 1939, 1943). These findings led to the conclusion that vagal input to the mammalian ventricle was both anatomically and functionally of little significance. Subsequently, anatomic (Jacobowitz et al., 1967; Kent et al., 1974), biochemical (Roskoski et al., 1974; Brown, 1976; Fields et al., 1978), and electrophysiological (for a review, see Rardon and Bailey, 1983a) evidence has been advanced suggesting a role for the parasympathetic nervous system in the regulation of ventricular electrical function. Despite the demonstration of all the elements necessary for parasympathetic influence in both atria and ventricle, the physiological basis for the difference in response to muscarinic cholinergic stimulation is not known. Since atrial myocardium receives a denser supply of parasympathetic input than the ventricle, it is possible that the observed difference in responsiveness to cholinergic stimuli is a function of the density of innervation. If so, alterations in the extent of cholinergic innervation may produce a change in the response of the tissue to cholinergic agonists. The following experiments tested the hypothesis that a chronic withdrawal of vagal input to the heart might influence the subsequent responsiveness of atrial and ventricular myocardium to acetylcholine applied in vitro.

Methods

Adult cats of either sex weighing 2.5–5 kg were utilized for these experiments. One group of cats served as nonoperated control animals, a second group underwent bilateral cervical vagotomy, and a third group underwent a sham operation. Cats undergoing bilateral cervical vagotomy were anesthetized with sodium secobarbital (30 mg/kg, ip); ECG lead 1 was monitored during surgery. Under sterile conditions the cervical vagi were isolated bilaterally and stimulated electrically through bipolar electrodes with a 10-V square wave 4 msec long, at a frequency of 10 Hz. The development of sinus slowing or atrioventricular (AV) block with vagal stimulation was demonstrated prior to nerve sectioning. A 5-mm segment of the vagosympathetic
posed to increasing concentrations of acetylcholine in the and cats after bilateral cervical vagotomy (BCV) was ex-
posed bilateral cervical vagotomy. In addition, action po-
tentials were recorded both from cats with intact vagi and
mine in cats with intact vagi, and in cats that had under-
We compared the atrial contractile response to physostig-
Previous work has shown that bilateral cervical vagotomy
of atrial tissue to physostigmine (10^-6 M) was examined.
transmembrane action potentials were recorded by standard microelectrode techniques. Action
potentials were displayed on a 5100 series Tektronix oscilloscope, and photographed with a Tektronix Polaroid oscilloscope camera. Single microelectrode impalements
were maintained for the duration of each experiment.
Tension was recorded from atrial trabeculae. One end
of the atrial muscle was pinned to the floor of the tissue bath, and the opposite end was attached by means of a stainless steel hook to a Gould Statham UC-3 strain gauge transducer fitted with a UL-5 arm. The strain gauge was
mounted on a micromanipulator allowing fine adjustment
of tissue length. Isometric tension was measured at the
length of 700 msec and ventricular fibers at a cycle length
of 1000 msec. Transmembrane action potentials were
recorded in the rate of developed tension (1100 ± 415 mg/
21). A reduction in
superfusate, and the responses were assessed. In separate experiments, the same concentration range of acetylcho-
line was applied in a random fashion. After all acetylcho-
lime concentrations had been administered, atropine was
applied concomitant with the highest concentration of acetylcholine.
The contribution of endogenously released catechol-
amines to the observed action potential changes was ex-
amined in two ways. In some experiments, the tissue was
exposed to propranolol (10^-6 m) either before or after
exposure to acetylcholine. This concentration of propran-
olol produces adrenergic blockade, but not direct mem-
brane effects (Wit et al., 1975). In separate experiments, cats
after bilateral cervical vagotomy were pretreated with
reserpine, 2 mg/kg, intraperitoneally, 24 hours before
sacifice. This dose has been shown to deplete tissue norepinephrine stores, as measured by a spectrophotosvo-
metric method (Dahlstrom and Haggendahl, 1966). In
addition, this dose of reserpine produces catecholamine
depletion sufficient to render tissues insensitive to super-
fusion with 10^-3 m tyramine, as previously shown (Rardon
and Bailey, 1983b).
To assess any possible role for changes in tissue levels of acetylcholinesterase, we superfused the atrial and ven-
tricular myocardium from cats with intact vagi, and cats
after bilateral cervical vagotomy, with solutions containing
carbamylcholine, a choline ester not hydrolyzed by acetylcholinesterase. Responses to carbamylcholine and
acetylcholine were compared to determine whether the
rate of hydrolysis of the agonist contributed to the ob-
served results.
Statistical comparison of dose responses in animals with
intact vagi and animals after bilateral cervical vagotomy
was made by a two-way analysis of variance with repeated
measures. When the analysis of variance was significant,
comparison of values from animals with intact vagi to
animals after bilateral cervical vagotomy was made by a
t-test adjusted for multiple comparison (Wallenstein,
1980). Paired t-tests were used if only one measurement
was compared to the control value.
Drugs used included atropine sulfate, acetylcholine
chloride, physostigmine sulfate, dl-propranolol hydro-
chloride, carbamylcholine, and tyramine hydrochloride,
all purchased from Sigma Chemical Company. Reserpin
was obtained from CIBA. Fresh solutions of each drug
were prepared daily.

Results
No significant differences were observed between the
responses of sham-operated cats or non-oper-
ated cats to the conditions of any of the experiments.
Data from these two groups have been pooled and
will hereafter be referred to as the group of cats with
intact vagi. In atrial muscle from cats with intact vagi, physostigmine (10^-4 m) produced a reduction in
developed tension of 30 ± 8% (266 ± 50 mg to
188 ± 43 mg, n = 7, P < 0.01), and a decrease in
the rate of developed tension of 33% (1814 ± 320
mg/sec to 1214 ± 415 mg/sec, n = 7, P < 0.01).
After bilateral cervical vagotomy, physostigmine
(10^-4 m) produced no significant change in de-
veloped tension (238 ± 82 mg to 227 ± 67 mg, n = 21)
or in the rate of developed tension (1100 ± 415 mg/
sec to 1207 ± 320 mg/sec, n = 21). A reduction in
atrial contractility of greater than 10% with physostigmine (10^{-6} M) was interpreted as an incomplete vagotomy. This occurred in less than 5% of the animals operated, and data from these animals were discarded.

In atria from cats with intact vagi, 10 minutes of exposure to physostigmine (10^{-6} M) increased resting membrane potential by 3 ± 1 mV and shortened action potential duration at 50% and 90% repolarization by 17 ± 5 msec and 31 ± 8 msec, respectively (n = 6). Action potential amplitude and V_{\text{max}} were unaffected by physostigmine. After bilateral cervical vagotomy, 10 minutes of exposure to physostigmine (10^{-6} M) produced no changes in atrial fiber resting membrane potential, action potential duration, action potential amplitude, or V_{\text{max}} (n = 6). In ventricular tissue from either cats with intact vagi (n = 5) or cats after bilateral cervical vagotomy (n = 6), physostigmine (10^{-6} M) failed to alter action potential duration, resting potential, action potential amplitude, or V_{\text{max}}.

The effects of exogenously applied acetylcholine were studied in atrial tissue from cats with intact vagi and cats after bilateral cervical vagotomy. In cats with intact vagi, acetylcholine increased resting membrane potential and shortened action potential duration. The effects of acetylcholine after bilateral cervical vagotomy were qualitatively similar, but the degree of action potential duration shortening was greater than in animals with intact vagi. No significant differences were observed in the magnitude of response between left and right atria, so data from these animals were pooled. Table 1 summarizes data from a series of these experiments. Membrane hyperpolarization occurred in response to acetylcholine in both groups. There were no significant differences between groups in the amount of hyperpolarization produced by acetylcholine. In cats after bilateral cervical vagotomy, acetylcholine produced a greater degree of shortening of the action potential duration measured at 50% and 90% repolarization. Figure 1 compares action potential duration responses in the two groups. Application of 10^{-8} M acetylcholine to fibers from cats after bilateral cervical vagotomy and from normally innervated cats failed to elicit a significant degree of action potential shortening.

Desensitization of the atrial tissue to the effects of acetylcholine did not occur with superfusion of a given concentration for up to 30 minutes. Randomizing the order of the applied concentrations of acetylcholine failed to alter the magnitude of the response to a given concentration of the agent. In all experiments, 10^{-6} M atropine reversed the effects of acetylcholine, indicating that the observed effects were mediated through muscarinic cholinergic receptors.

The action potential characteristics of ventricular myocardium prior to the administration of any drugs were similar in the control animals and animals after bilateral cervical vagotomy. No statistically significant differences were observed between the two groups in action potential amplitude, action potential duration at 50% and 90% repolarization, resting potential, or V_{\text{max}}. Right ventricular papillary muscles or left ventricular muscle strips from control animals showed no changes in any of the measured action potential characteristics on exposure to exogenous acetylcholine (10^{-7} to 10^{-5} M). In right ventricular papillary muscles and left ventricular muscle strips from cats after bilateral cervical vagotomy, acetylcholine shortened action potential duration at both 50% and 90% repolarization (Fig. 2). The effects of acetylcholine were concentration dependent, with effects first apparent at a concentration of 10^{-7} M. Application of doses of acetylcholine greater than 10^{-5} M failed to elicit a greater response. Acetylcholine did not affect resting membrane po-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Action Potential Characteristics in Cat Atria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine concentration</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cats with intact vagi (n = 6)</td>
</tr>
<tr>
<td>APA (mV)</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>RP (mV)</td>
<td>76 ± 8</td>
</tr>
<tr>
<td>APD_{50} (msec)</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>APD_{90} (msec)</td>
<td>138 ± 6</td>
</tr>
<tr>
<td>V_{\text{max}} (V/sec)</td>
<td>240 ± 100</td>
</tr>
<tr>
<td></td>
<td>Cats after bilateral cervical vagotomy (n = 6)</td>
</tr>
<tr>
<td>AP (mV)</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>RP (mV)</td>
<td>77 ± 6</td>
</tr>
<tr>
<td>APD_{50} (msec)</td>
<td>63 ± 5</td>
</tr>
<tr>
<td>APD_{90} (msec)</td>
<td>146 ± 7</td>
</tr>
<tr>
<td>V_{\text{max}} (V/sec)</td>
<td>200 ± 70</td>
</tr>
</tbody>
</table>

Results are expressed as means ± s. APA = action potential amplitude; RP = resting potential; APD_{50} and APD_{90} = action potential duration at 50% and 90% repolarization, respectively.

* Significantly different from acetylcholine concentration = 0, P < 0.01.
FIGURE 1. Comparison of action potential duration shortening produced in atria from animals with intact vagi (●, ■), and animals after bilateral cervical vagotomy (BCV) (○, □). Action potential duration (APD) is plotted as function of acetylcholine (ACh) concentration. The upper pair of curves represents values for APD at 90% repolarization, and the lower pair of curves represents values for APD at 50% repolarization. An (*) indicates a significant (P < 0.01) difference from the value for APD at 0 concentration of ACh within that group. Differences in the response to ACh between the two groups were analyzed by two-way ANOVA with repeated measures. There is a significant difference in APD response to ACh between cats with intact vagi and cats after BCV when measured at 90% repolarization (interaction F = 7.88, P < 0.001) and 50% repolarization (interaction F = 16.4, P < 0.001). This exaggerated effect of ACh is graphically illustrated by the increased steepness of the curve for animals after BCV.

FIGURE 2. The effect of ACh on action potentials in ventricular muscle from a cat after BCV. The preparation is paced at a cycle length of 1000 msec. Values for APD at 50% and 90% repolarization are shown within each action potential. Panel A shows the control action potential. Panel B: application of 10⁻⁷ M ACh. Panel C: application of 10⁻⁶ M ACh. Further shortening occurs with the application of 10⁻⁵ M ACh in panel D. Vertical calibration bar equals 40 mV. Horizontal calibration bar equals 50 msec.

Discussion

Our present experiments indicate that chronic vagotomy alters the response of both atrial and ventricular muscle to cholinergic stimuli. These responses are mediated through muscarinic cholinergic receptors, and are direct effects—that is, effects independent of the level of adrenergic tone. Vagal stimulation or the application of muscarinic cholinergic agonists alters the contractile (Webb, 1950; Sarnoff et al., 1960) and electrical (Hoffman and Suckling, 1953) properties of the mammalian atrium. More recently, vagal stimulation or muscarinic cholinergic agonists have been shown to alter the mechanical (DeGeest et al., 1965; Levy et al.,...
by guest on October 26, 2017

alters ventricular refractoriness (Blair et al., 1980; man, as well (Prystowsky et al., 1981). Conversely, a significant effect on ventricular refractoriness in Martins and Zipes, 1980), and vagal tone may exert stimulation of vagal nerves in the experimental animal (Car- meliet and Ramon, 1980; Iipsius and Gibbons, 1983), on exposure to acetylcholine. Stimulation of vagal nerves in the experimental animal alters ventricular refractoriness (Blair et al., 1980; Martins and Zipes, 1980), and vagal tone may exert a significant effect on ventricular refractoriness in man, as well (Prystowsky et al., 1981). Conversely, ablation of specific parasympathetic fibers to the heart can eliminate the effects of truncal vagal stimulation on atrial and ventricular contractile response (Randall et al., 1983). Thus, either augmentation or withdrawal of vagal input can influence cardiac function.

After complete interruption of vagal input, a rapid decrease in the biochemical markers of parasympathetic activity occurs (Brown, 1976; Lund et al., 1978, 1979). The fact that the application of physostigmine to atrial tissue following bilateral cervical vagotomy produces no effect provides a physiological correlate for the observation that vagotomy depletes tissue stores of acetylcholine.

After bilateral cervical vagotomy, the effect of acetylcholine on atrial action potential duration is exaggerated. We define exaggerated to mean that, although the threshold concentration necessary to elicit a response does not appear to be changed, the magnitude of the response is increased. This is in contrast to the classical supersensitivity of denervated structures to neurotransmitters, in which the threshold concentration is decreased, but the maximum effect is unchanged (Cannon and Rosenblueth, 1936).

No conclusions can be rendered from these experiments concerning the changes in ionic currents.
and Pappano, 1980). Although the results of our ventricular experiments could be due to this type of interaction, the effects of acetylcholine remained intact with β-receptor blockade in vitro, or with depletion of endogenous catecholamines prior to sacrifice. These results lead us to conclude that the observed effects of acetylcholine in the ventricle were due to direct effects of muscarinic cholinergic stimulation. The demonstration of direct muscarinic cholinergic effects on action potential duration in ventricular muscle is important in further defining the functional significance of parasympathetic innervation of the ventricle. Despite multiple lines of evidence demonstrating vagal input to mammalian ventricle, direct effects of muscarinic cholinergic agonists on ventricular action potential characteristics have been demonstrated in only one species, the guinea pig (Ochi and Hino, 1978). Our data suggest that, at least under the specific conditions of our experiments, the cellular and subcellular components necessary to translate muscarinic cholinergic stimuli into a direct effect on cellular action potentials exist in mammalian ventricular myocardium.

Altered sensitivity to acetylcholine after bilateral cervical vagotomy could occur through several mechanisms. Decreased hydrolysis of acetylcholine responsible for the observed change in action potential duration of atrial and ventricular muscle. However, the effects of acetylcholine on normally innervated atrial muscle are mediated through an increase in membrane potassium conductance (Trautwein et al., 1956); thus an exaggerated effect of acetylcholine could be expected to be mediated through this mechanism. It is interesting to note that the hyperpolarization of resting membrane potential produced by acetylcholine was not significantly changed after bilateral cervical vagotomy. The dissociation of these two manifestations of altered potassium conductance may suggest that altered sensitivity to acetylcholine is not simply an amplification of the normal increase in potassium flux seen in cholinergic stimulation.

Previous studies have shown that enhanced sensitivity to acetylcholine may be seen in the presence of simultaneous adrenergic stimulation (Levy, 1971; Inui and Imamura, 1977; Bailey et al., 1979; Biegon

![Figure 3. A comparison of the effect of carbamylcholine, a nonhydrolyzed choline ester, and ACh on atrial action potential duration. The top panel illustrates values from cats with intact vagi. There is no significant difference in the action potential duration shortening produced by the two drugs. The bottom panel illustrates values from cats after BCV. Note first that there is an exaggerated response to each cholinergic agonist when compared to the cats with intact vagi. In addition, there is no significant difference in the action potential duration shortening produced by the two drugs in cats after BCV.](http://circres.ahajournals.org/)

![Figure 4. A comparison of the effect of carbamylcholine and acetylcholine on ventricular action potential duration. Top panel illustrates values from cats with intact vagi. Neither agonist produces a significant effect on APD. The bottom panel illustrates values from cats after BCV. Both agonists shorten APD. There was no statistically significant difference in the APD shortening produced by the two drugs in cats after BCV.](http://circres.ahajournals.org/)
following bilateral cervical vagotomy could increase the functional concentration of acetylcholine at its receptor and produce a greater physiological response. This possibility is unlikely, due to the fact that carbamylcholine produces the same exaggerated effects on action potential duration as does acetylcholine. Thus, the effects of muscarinic cholinergic agonists after bilateral cervical vagotomy are not dependent on the rate of hydrolysis of acetylcholine.

Complications related to the surgical procedure could be the cause for altered sensitivity to acetylcholine after bilateral cervical vagotomy. Two possible complications of a neck dissection, hypothyroidism and hypoxia, have been shown to alter the number of muscarinic cholinergic receptors in the heart as measured by radioligand-binding techniques (Sharma and Banerjee, 1977; Crockatt et al., 1981). It is unlikely that the consequences of a surgical procedure other than vagotomy are responsible for our results, however, since sham-operated animals responded to acetylcholine in a manner identical to nonoperated animals.

Denervation supersensitivity following vagal transaction is another possible mechanism. Organs become supersensitive to their neurotransmitters after denervation (Cannon, 1949). Although this is an attractive hypothesis, data from other investigators would argue against this interpretation of our experiments. The denervated mammalian heart shows supersensitivity to exogenous catecholamines (Cooper, 1965; Spann et al., 1966; Dempsey and Cooper, 1968). However, both total cardiac denervation and selective parasympathectomy of the heart fail to produce supersensitivity to the effects of acetylcholine on contractility or heart rate in vivo (Jacobs et al., 1974; Hageman et al., 1975; Priola and Spurgeon, 1977). The heart may be an exception to Cannon’s "law of denervation," since extrinsic cardiac denervation does not alter the intrinsic post-ganglionic parasympathetic neurons (Priola et al., 1977; Priola and Spurgeon, 1977), leaving the heart decentralized but not denervated with respect to this limb of the autonomic nervous system. Bilateral cervical vagotomy also decentralizes the heart from parasympathetic influence, leaving the intrinsic nerves intact. A failure to denervate the heart does not completely eliminate supersensitivity as an explanation for our results, however, since organs can show an increase in sensitivity to neurotransmitter following decentralization (Cannon and Rosenblueth, 1936).

Denervation or decentralization supersensitivity might be expected to be due to an increase in muscarinic receptor number or affinity at the cell surface. Although chronic exposure to cholinergic agonists has been shown to decrease the receptor number in cultured cells (Haltwisher and Nathanson, 1981), receptor numbers have not been determined in the heart following withdrawal of cholinergic tone. In addition, simple determination of receptor numbers may not be sufficient to determine whether the receptors are capable of mediating a physiological response at the cell level. Thus, chronic bilateral cervical vagotomy in the cat could alter responsiveness to acetylcholine at the level of the receptor, at the level of receptor effector coupling, or at both levels.

References

Cooper T (1965) Physiologic and pharmacologic effects of cardiac denervation (abstr). Fed Proc 24: 1428
Hageman GR, Urthaler F, James TN (1975) Absence of supersensitivity to acetylcholine in the extrinsically denervated sinus

We wish to acknowledge the excellent technical assistance of David Mendel and the excellent secretarial work of Carmen Mason and Christy Jones.

Supported in part by the Herman C. Kranert Fund, and by Grants HL06308 and HL07182 from the National Heart, Lung, and Blood Institute, National Institutes of Health.

Dr. Kovacs is a U.S. Public Health Service Trainee in Cardiology. Address for reprints: John C. Bailey, M.D., Indiana University School of Medicine, Kranert Institute of Cardiology, 1001 W. 10th Street, Indianapolis, Indiana 46202.

Received April 26, 1984; accepted for publication December 20, 1984.
INDEX TERMS: Acetylcholine • Vagus nerve • Electrophysiology • Muscarinic receptor
Effects of acetylcholine on action potential characteristics of atrial and ventricular myocardium after bilateral cervical vagotomy in the cat.
R J Kovacs and J C Bailey

Circ Res. 1985;56:613-620
doi: 10.1161/01.RES.56.4.613

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/56/4/613

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

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

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