Cholinergic Mechanisms in the Sinus Node

WITH PARTICULAR REFERENCE TO THE ACTIONS OF HEMICHOLINIUM

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ABSTRACT

Acetylcholine is directly perfused into the canine sinus node in vivo through its cannulated nutrient artery. After atropinization, the chronotropic action from intranodal administration of acetylcholine was identical to that of control injections of Ringer's solution alone. Eserine produced only a negative chronotropic action before, and no significant action after, atropinization. Direct perfusion of the sinus node with hemicholinium (1 mg/ml) completely blocked the response to vagal stimulation but had no effect on the response to stimulation of the stellate ganglion. The evidence is interpreted as indicating that acetylcholine has only a negative chronotropic action in the canine sinus node and that it neither releases significant amounts of local nodal norepinephrine nor is necessary to the normal response of the sinus node to stellate ganglion stimulation.

ADDITIONAL KEY WORDS acetylcholine eserine atropine chronotropic effect hemicholinium chronotropic effect acetylcholine hemicholinium and adrenergic neurotransmission acetylcholine and adrenergic neurotransmission direct perfusion of sinus node anesthetized dog

Vagal slowing of the heart is one of the oldest and most familiar experiments in the biological laboratory. In recent years two pharmacologic advances have raised some important questions regarding the precise nature of cholinergic mechanisms in the normal pacemaker of the heart. The first of these is the concept proposed by Burn and Rand1 that acetylcholine is not only essential in postsynaptic transmission from cholinergic nerves, but that its initial release is also essential for the subsequent liberation of norepinephrine from adrenergic nerve terminals. The second is the discovery of the hemicholiniums by Schueler2 and the subsequent demonstration by MacIntosh, Birks and Sastry3 that hemicholinium-3 (HC3) blocked the local synthesis of acetylcholine within neurons by inhibiting choline acetylase and thus interrupting the transport of choline into the cells.

An alternative explanation for certain aspects of the hypothesis of Burn and Rand has been suggested by Leaders,4 based on the chronotropic action of adrenergic and cholinergic nerve stimuli delivered to isolated atria of cats before and after reserpine and hemicholinium. Leaders' evidence indicates that either adrenergic or cholinergic nerve stimuli can influence the other's postsynaptic transmission without the necessary participation of acetylcholine in the neural release of myocardial norepinephrine. We have employed a method5 for direct perfusion of the sinus node of the dog in vivo through its nutrient artery to study the pharmacology of a variety of substances, with particular attention to their "pure" chronotropic action as well as their secondary effects on either cholinergic or adrenergic neural transmission.6,8 The present experiments were designed to study the local action of acetylcholine during direct perfusion of the sinus node, particularly for evidence of norepinephrine release when acetylcholine was administered in low concentrations or after atropinization. The intranodal actions of eserine were similarly studied. Finally, the
pharmacologic effect of intranodal hemicholinium-3 on the response of the sinus node to vagal stimulation, intranodal acetylcholine and stellate ganglion stimulation was examined.

Methods

Dogs weighing 10 to 20 kg were anesthetized with pentobarbital (30 mg/kg), and the chest was opened in the midline to suspend the heart in the pericardium. Through a tracheotomy, ventilation with room air was performed with a Harvard pump. The right coronary artery was dissected free in the atrioventricular sulcus and, after ligation, was cannulated with a polyethylene tube, which was then passed up the sinus node branch and secured with a ligature. Ligation of the canine sinus node artery has no significant effect on sinus rhythm, largely because of the extensive arterial anastomoses there. A three-way stopcock and transducer were attached to the proximal end of the cannula in the sinus node artery to measure retrograde pressure and to permit injections into the artery. All injections were from a hand syringe and usually of 2-ml volume delivered in about 10 sec; longer injections and larger volumes were occasionally used, as indicated. Cannulae were also placed in the right atrium via the jugular vein and in the central aorta via a femoral artery. These pressures and the one in the sinus node artery were recorded on a polygraph simultaneously with an ECG and a tachograph derived from successive R waves. Because most recording on the polygraph was at slow speed (0.25 mm/sec) a simultaneous ECG was inscribed separately through a slave circuit on a single-channel recorder at 25 or 50 mm/sec throughout the experiments and collated with the master record; this tracing permitted accurate definition of ECG complexes and intervals. Pulses and the ECG were monitored on a four-channel oscilloscope during each experiment at a sweep speed of 50 mm/sec.

The right vagus nerve was isolated in the neck and the right stellate ganglion within the thorax for stimulation with an electronic square-wave stimulator at supramaximal voltage, 30 cps, 1 msec impulses in 6-sec trains. Test materials were prepared in Ringer's solution and were always compared with control injections of Ringer's solution alone. Any injection directly into the sinus node artery (including fresh autogenous arterial blood) produces a transient bradycardia followed by a brief postinjection acceleration; the slowing is not due to temperature, pH, ion content of injectate or a neural stimulus, but is most likely a physical response to distention of the sinus node artery. The postinjection acceleration can be blocked with beta-receptor blocking agents and is most likely due to transient release of local norepinephrine by the injection. Substances examined in the present experiments were: acetylcholine hydrochloride, eserine salicylate, hemicholinium-3 dibromide, 1-norepinephrine bitartrate and atropine sulfate.

Results

Acetylcholine produced only one type of chronotropic action in 25 dogs and that was slowing of the sinus node (fig. 1). Concentrations between 0.0001 and 0.01 μg/ml had an effect identical to that of Ringer's solution alone in most dogs, although slight inconsistent slowing was produced at 0.01 μg/ml in 8 dogs. Concentrations of 0.1 μg/ml always produced marked slowing (over 50% below control rate) and usually sinus arrest with an escape A-V nodal rhythm. Concentrations of 1.0 μg/ml always produced sinus arrest and in about 25% of instances there was associated transient atrial fibrillation. The postinjection acceleration following the lesser concentrations of acetylcholine was the same as that following Ringer's solution; following the transient hypotension during marked bradycardia with greater concentrations of acetylcholine, there was both slight hypertension and tachycardia after the resumption of sinus rhythm.

It was presumed that slight cardiac acceleration after hypotension due to bradycardia was caused by reflex release of catecholamines from the adrenal medulla, or by their release directly from the medulla by undestroyed acetylcholine recirculating after passing through the sinus node. Because identical volumes of acetylcholine injected intravenously failed to produce tachycardia, the latter explanation was unlikely. To test the possibility that either dilute or high concentrations of acetylcholine did release local nodal norepinephrine, the action of which might be obscured by the negative chronotropic effect of acetylcholine, acetylcholine in concentrations from 0.001 to 1.0 μg/ml was injected in 10 dogs after local nodal atropinization and after intravenous atropinization in 5 others. For local nodal atropinization, 10 μg/ml produces effective cholinergic block-
intravenous atropine was given as 1 mg/kg. Acetylcholine at all concentrations tested had no significant chronotropic action after atropinization in either manner. The maximal slowing during injection and maximal postinjection acceleration were identical to those from control Ringer’s injections in the same dogs. Possible exploration of the

**FIGURE 1**

Polygraphs representing typical responses to increasing concentrations of acetylcholine (ACh) injected directly into the sinus node artery before and after atropinization (ATR). The responses were the same whether atropinization was selectively nodal (10 μg/ml) or intravenous (1 mg/kg). The control response to Ringer’s solution alone (R) is bradycardia during injection followed by a brief postinjection acceleration (see text). The only chronotropic response attributable to acetylcholine at any concentration before atropine is slowing of the sinus node. After atropinization all concentrations of acetylcholine had effects identical to those of Ringer’s solution alone. The last injection in B is norepinephrine (NE), given for comparative purposes; the asterisk indicates a brief artefact in the tachogram. In C the sequence of norepinephrine and acetylcholine injections is reversed, with intervening injections of Ringer’s solution, both to clear the preceding norepinephrine (first Ringer’s) and to demonstrate control responses (next two Ringer’s). Recordings from above down represent right atrial pressure (RA), retrograde pressure in the cannulated ligated sinus node artery (SNA), central aortic pressure (Ao), a tachogram reflecting beat-to-beat heart rate (HR) and the ECG (here AVR). Pressures are scaled in millimeters Hg and heart rate in beats per minute. All three of these panels are from the same dog. Recordings and labels in subsequent polygraph illustrations are the same as these.
effect of beta-receptor blocking agents in the same experiments is precluded by the demonstration that these substances have not only adrenergic but also cholinergic blocking actions on direct perfusion of the sinus node. Although vagal stimulation may produce sinus acceleration after intranodal atropinization, the cervical vagus contains adrenergic fibers which may account for this action without requiring the participation of acetylcholine.

Since it was possible that brief injections of acetylcholine released a small amount of norepinephrine which exerted an action inapparent because of brevity, longer injections of acetylcholine (30 to 60 sec) were performed. Before atropinization, only results from the lower concentrations of acetylcholine could be interpreted for direct chronotropic action because those of 0.01 µg/ml and greater produced so much slowing and hypotension that extracardiac compensatory mechanisms became prominent factors. The lower concentrations of acetylcholine with longer perfusion still acted exactly as did Ringer’s solution alone injected for the same period of time. Concentrations of 0.01 to 1.0 µg/ml acetylcholine perfused into the sinus node after atropine also produced the same effect.

**FIGURE 2**

Strips of ECG recorded during right vagal stimulation (RV) before (C) and at regular intervals after injection of hexamethonium into the sinus node artery, demonstrating selective complete vagal blockade in the sinus node immediately after hexamethonium (HC), followed by gradual recovery of the normal response. Both the decreasing number of escape sinus beats (P waves) and the progressively later time of the first escape show gradual waning of the vagal blockade. During selective blockade of the response by the sinus node there are no transmitted QRS complexes since the A-V node was not perfused with hexamethonium. All strips are of lead AVR, recorded at 25 mm/sec and sensitivity of 10 mm for 1 mv.
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as Ringer's solution alone. No bouts of atrial fibrillation occurred after atropinization with any concentration of acetylcholine perfused either briefly or for a longer time.

In a previous study we had observed that low concentrations of intranodal eserine produced some sinus acceleration after atropinization. Since inhibition of cholinesterase and accumulation of nodal acetylcholine may have in turn led to sustained norepinephrine release, this possibility was re-examined in 8 dogs. All these dogs received standard 10-sec injections but in 4 eserine was perfused for 30 sec both before and after atropinization. As previously observed, the sinus bradycardia from eserine was gradual in onset and of prolonged duration. In 5 dogs, intranodal eserine (1.0 µg/ml) produced sinus bradycardia 20 to 40% below the control heart rate, which lasted 10 to 20 minutes. At 10 µg/ml, eserine produced more marked sinus bradycardia and transient sinus arrest with escape A-V nodal rhythm in all 8 dogs; this slowing lasted 20 to 30 minutes. Neither brief nor prolonged nodal perfusions produced sinus acceleration at any concentration studied, before or after atropinization.

Hemicholinium (0.01 to 1,000 µg/ml) was injected into the sinus node of 6 dogs. Below 100 µg/ml there was no significant direct chronotropic action and only inconsistent inhibition of vagal slowing. At 100 µg/ml there was temporary partial inhibition of vagal slowing. At 1,000 µg/ml there was consistent complete vagal blockade, which gradually waned over 20 to 30 min (figs. 2 and 3);

FIGURE 3

Strips of ECG demonstrating a comparison of selective vagal blockade in the sinus node produced by hemicholinium and by atropine. These strips are not in sequence, but all are from the same dog as those in figure 2. A is a control response to vagal stimulation; B, the response to an identical stimulus 2 min after an intranodal injection of atropine (10 µg/ml). C demonstrates complete blockade of the response to a concentration of intranodal acetylcholine which had previously produced immediate sinus arrest (see fig. 6). An injection of Ringer's solution alone in D produces a response identical to the preceding acetylcholine. The vagal blockade by atropine was allowed to recover, with sinus arrest again being the response. Then hemicholinium (1 mg/ml) was injected into the sinus node artery and the response to vagal stimulation (E) shows selective vagal blockade virtually identical to that produced by intranodal atropine. F is for comparative purpose and demonstrates the response to vagal stimulation after intravenous atropinization with 1 mg/kg; the difference from selective vagal blockade in the sinus node is that now all P waves are transmitted to produce QRS.
The characteristic response to intranodal eserine, its selective reversal by intranodal hemicholinium and the subsequent blockade of eserine effect are shown in these polygraphs. In A, 1 μg/ml of eserine produced a response identical to control injections of Ringer's solution, while 10 μg/ml produced gradual onset of sinus bradycardia which lasted 18 min before return of HR to control level; an intervening injection of Ringer's solution (R) produced transient A-V nodal rhythm, which is apparent in the atrial pulse, but had no significant effect on the basic response to eserine or its duration on comparison to previous responses without post-esserine injections. The Ringer's injection response may be compared to the response to intranodal hemicholinium shown in B and injected at the same interval after eserine; there is prompt reversal of the sinus bradycardia from eserine. In C, eserine is injected immediately after a later hemicholinium injection and its effect is blocked.
during this time there was an associated sinus acceleration identical to that observed with vagal-blocking concentrations of atropine. Although 1,000 μg/ml is a relatively high concentration of hemicholinium, it was necessary to produce consistent vagal blockade. More prolonged local intranodal administration than that employed in the present study is probably needed to produce vagal blockade with lower concentrations of hemicholinium.

When administered during the sinus bradycardia from intranodal eserine, hemicholinium produced prompt partial reversal of the bradycardia at 100 μg/ml in 3 dogs and complete reversal at 1,000 μg/ml in 5 dogs (figs. 4 and 5). Intranodal eserine (10 μg/ml) had no significant chronotropic action within the first 10 min after intranodal hemicholinium (1,000 μg/ml). Both the reversing and blocking actions were identical to those of atropine against eserine. Intranal acetylcholine (0.1 μg/ml) was injected at 2- to 4-min intervals after hemicholinium (1,000 μg/ml) in 5 dogs; it had produced abrupt sinus arrest in all 5 prior to hemicholinium; in 3 the slowing from acetylcholine was blocked for 2 to 8 min (fig. 6), but the effect of intranodal acetylcholine was unaltered in the other 2 dogs. Vagal blockade was complete in all 5, and in the 3 with inhibition of intranodal acetylcholine action the vagal blockade lasted over three times as long as the acetylcholine block or antagonism.

If acetylcholine is an essential component
of the postsynaptic neurotransmitter release in adrenergic nerve terminals, interruption of local acetylcholine synthesis with intranodal hemicholinium should impair the response of the sinus node to stellate ganglion stimulation. In 4 dogs the response to stellate stimulation was compared before and after intranodal hemicholinium (1,000 μg/ml). The accelerative response was the same (fig. 7). Comparative responses to vagal stimulation demonstrated that there was complete vagal blockade in the same dogs at those times. Because stellate stimulation after intranodal beta-receptor blockade produces A-V nodal tachycardia, the ECG was examined to be sure the cardiac acceleration from stellate stimulation after hemicholinium originated from the same pacemaking site, and the P waves were identical to those from the control period (fig. 8).

Discussion

Three lines of evidence indicate that acetylcholine does not release and is not essential for the release of norepinephrine in the canine sinus node in situ. The first of these is the response to acetylcholine itself: neither single injections at dilute concentrations nor relatively prolonged perfusion caused any significant acceleration, and after atropinization the response to all concentrations of acetylcholine used at all perfusion times was identical to that of control injections of Ringer's solution alone. The second is the absence of acceleration from intranodal eserin at low concentrations, after short or longer perfusion
An electrogram recorded in the same manner as figure 5 (same dog) is shown here during two of the stellate stimulus responses in figure 7. Duration of the stimulus is apparent from its artefact in the recording, but is also indicated by a horizontal bar. Three strips beginning at A are continuous and demonstrate the sinus acceleration produced by a control stimulus (RS, C); P-wave contour remains stable and PR interval about the same during the tachycardia. In B the first stellate stimulus after hemicholinium in figure 7 is recorded to demonstrate the pacemaker site is the same as during the control stimulus. C demonstrates the vagal blockade present 4 min after a later injection of hemicholinium in the same dog, during a series in which stellate and vagal stimuli were alternated at 2-min intervals. There is complete vagal blockade at a time when the stellate stimulus response was unchanged.

times, or after atropinization. Previous observations on effects of intranodal eserine showed that a slight sinus acceleration resulted when the drug was administered in low concentrations; these results are not confirmed. The third line of evidence is the identical accelerative response to stellate stimulation both before and after intranodal hemicholinium in concentrations which produced complete vagal blockade and presumably complete inhibition of local acetylcholine synthesis.

These observations fail to support the hypothesis that acetylcholine release is a necessary precursor to norepinephrine release at postsynaptic adrenergic nerve terminals. Some time ago Burn suggested, however, that certain aspects of the hypothesis may not apply to structures which are primarily vascular. In this respect it is important to note that the sinus node in man and the dog is in essence a periarterial structure and has been aptly described as resembling an enormous adventitia of its artery. Some of Leaders’ evidence against the necessary participation of acetylcholine in postsynaptic release of norepinephrine from adrenergic nerves was also obtained in vascular tissue. However, Leaders and Dayrit found hemicholinium also failed to inhibit sympathetic nerve transmission in tissue not primarily vascular (spleen).
The possibility that intranodal acetylcholine does release minute concentrations of norepinephrine within the sinus node has not been excluded, although the significance of such a possibility is made negligible in the absence of any demonstrable acceleration of the node. It is possible, for example, that intranodal perfusion of acetylcholine releases very small amounts of norepinephrine during the perfusion (just as Ringer's solution alone may) but that this is degraded rapidly and its action inapparent at the end of the injection. Several points suggest this is not the case. If norepinephrine was released by acetylcholine, the injection bradycardia should have been lessened with increasing concentrations of acetylcholine as it is with injection of norepinephrine itself. Similarly, since the effect of intranodal norepinephrine in concentrations sufficient to produce acceleration lasts 1 to 3 min after the injection is complete, it seems unlikely that intranodal degradation or inactivation of norepinephrine is so rapid that action of any released by acetylcholine would be inapparent, particularly with the administration of relatively large concentrations of either acetylcholine or eserine after atropine.

Hemicholinium reversed the negative chronotropic action of intranodal eserine with the same speed as did atropine. Since the speed of onset of action by hemicholinium in blocking acetylcholine synthesis is rapid (10 to 20 sec), lack of destruction of accumulated acetylcholine must not be a major component of the negative chronotropic action of intranodal eserine, since any accumulated undestroyed acetylcholine should have continued to keep the sinus node slow for some time after the termination by hemicholinium of local acetylcholine synthesis. Bradycardia from eserine must depend on constant acetylcholine synthesis, in order to explain it according to most concepts of the mechanisms of action of eserine. On the other hand, if one presumed that some of the action of eserine was direct and independent of acetylcholine, then this action too must be susceptible to prompt reversal and blockade by hemicholinium. It seems more likely for the present that the chronotropic action of eserine does rely on continued acetylcholine synthesis and inhibition of its breakdown by cholinesterase, but that inhibition of cholinesterase quickly becomes unimportant in the sinus node once acetylcholine synthesis is interrupted.

Both adrenergic and cholinergic nerve terminals are present in the canine sinus node and in addition there are many juxtanodal cholinergic ganglia. It is possible that some of the vagal blocking action of hemicholinium, a bisquaternary ammonium compound, may have occurred within the ganglia. However, in previous experiments with direct perfusion of the sinus node, the actions of hexamethonium and veratridine have been neither as consistent nor as prolonged as that of atropine, and the vagal blocking of hemicholinium was similar to that of atropine. The unexpected observation of diminished effectiveness of intranodal acetylcholine for a short period after hemicholinium is difficult to explain. A nonspecific "anesthetic" effect within the sinus node by these high concentrations of hemicholinium is possible, although the persistence of sinus acceleration from stellate ganglion stimulation is evidence against such a nonspecific action. A more tenable hypothesis is that hemicholinium, at least transiently, combines with the same receptor sites employed by acetylcholine. Such combination at the receptor sites must not last as long as inhibition of cholinesterase by hemicholinium, however, since vagal blockade regularly outlasted and was more consistent than inhibition of the response to intranodal acetylcholine.

Acknowledgment

I am grateful to Dr. Vernon B. Haarstad, Department of Pharmacology, Tulane University, School of Medicine, New Orleans, La., who supplied me with Hemicholinium no. 3 dibromide (Lot No. V2-77-1).

References

Cholinergic Mechanisms in the Sinus Node: With Particular Reference to The Actions of Hemicholinium
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Circ Res. 1966;19:347-357
doi: 10.1161/01.RES.19.2.347

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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