Effects of ionic channel antagonists barium, cesium, and UL-FS-49 on vagal slowing of atrial rate in dogs

DON W. WALLICK, AKIN KUGUOGLU, TIANEN YANG, SHERRY L. STUESSE, AND MATTHEW N. LEVY
Division of Investigative Medicine, Mt. Sinai Medical Center, Cleveland 44106; and Department of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

METHODS

Surgical preparation. Eighteen mongrel dogs of either sex (18–24 kg) were premedicated with morphine sulfate (2 mg/kg im) and anesthetized with α-chloralose (100 mg/kg iv) 30 min later. A tracheal cannula was inserted, and intermittent positive-pressure ventilation was begun. The chest was opened transversely at the fourth intercostal space. The pericardium was incised, and the heart was placed in a cradle. The atrial electrogram (A wave) was recorded from a bipolar plate electrode sutured onto the right atrial appendage. We calculated the atrial cycle length from the atrial electrogram by use of a custom analog computer. We measured the blood pressure in a femoral artery by means of a Statham pressure transducer (Gould, Cleveland, OH). The atrial electrograms, atrial cycle lengths, and arterial blood pressure were recorded on an eight-channel oscillograph (Gould TA 2000). Both cervical vagi were crushed with ligatures to interrupt neural conduction. A pair of stainless steel plunge electrodes (0.2 mm, insulated to within 2 mm of the tip) was inserted into the right vagus nerve caudal to the ligature. The electrodes were connected to a constant-current isolation unit. The stimulation placement was determined by our custom software described in Vagal stimulation paradigm. Stimulation through such plunge electrodes evokes stable responses over many hours (17). The right and left stellate ganglia were isolated. The upper poles of both stellate ganglia were crushed by tight ligatures to interrupt tonic sympathetic impulses to the heart (19).

Vagal stimulation paradigm. With our IBM-compatible computer, a Lab 40 real-time data-acquisition system (Continuum system), and our custom-written software, we applied a single burst of stimulation to the right cervical vagus nerve (1-ms pulse duration, 10 pulses/burst, 10-ms interpulse interval, 1 mA). Brief electrical bursts were applied to the right vagus nerve once every minute at a precise delay after the atrial depolarization. The 1-min time frame allowed the nerve and SA node to recover between stimuli. The delays of stimulation were incremented by 50 ms up to the prevailing atrial cycle length before the application of vagal stimulation. The results were printed out on-line. After visually inspecting the results, we applied single bursts at delays in-between some of the 50-ms increments, in the portion of the curve

0363-6135/97 $5.00 Copyright © 1997 the American Physiological Society
Effect curves were generated. The final concentration for each was adjusted to cause a 100-ms increase in the resting atrial cycle length. Barium chloride (50 mM) was diluted in the perfusate before entering the SA nodal artery. The concentration of barium was adjusted to cause a 100-ms increase in the resting atrial cycle length and was continued during the time that vagal-effect curves were generated. The final concentration for each dog ranged from 0.25 to 1 mM. Fifteen minutes after the perfusion was begun, the vagus nerve was stimulated as described in Vagal stimulation paradigm. Higher concentrations of barium that further increased the resting atrial cycle length caused arrhythmia, and thus higher concentrations were not used (based on four pilot experiments). In the second group of dogs (n = 6), cesium chloride was infused into the perfusate. We adjusted the cesium perfusate rates based on the desired final concentration (0.5, 1.0, or 2.0 mM). A concentration of 1.0 mM completely blocks the I_f in a rabbit SA preparation, whereas doses > 5 mM block other currents (4). At each concentration, we waited 15 min after beginning the perfusion before generating a new vagal-effect curve. In the third group of dogs (n = 5), we gave an intravenous dose of 0.05 mg/kg of UL-FS-49 and waited 15 min before generating a vagal-effect curve. A second 0.05 mg/kg dose was then administered. After a second 15-min wait, the stimulations were repeated.

RESULTS

A single vagal volley causes complex changes in sinus rhythm (Figs. 1 and 2). The changes in rhythm result in an overall triphasic response that consists of two periods of increased atrial cycle length (Fig. 2A, I and II) separated by a transient period of acceleration (Fig. 2A, ac). In some cases, the heart rate returns close to the control level but does not actually accelerate between the two periods of slowing. However, for the purpose of description, we will call the response an acceleration. To illustrate in one dog, if the stimulus is delayed by 350 ms (Fig. 1B), the cycle in which the stimulus is given is not prolonged, but the subsequent cycle is increased to almost 1,400 ms. However, a 300-ms vagal stimulation delay immediately prolongs the A-A interval to >1,600 ms versus a prestimulation cycle length of 600 ms (Fig. 1A). By 12–14 s poststimulation, the A-A interval has returned to the control (prestimulation) level. If many of these responses are plotted together, one obtains a “vagal-effect curve” (Fig. 2A).

Barium, an I_{K,ACh} channel antagonist. An example of an experiment in which we used barium as the antagonist is shown in Fig. 2. In the absence of barium, vagal stimuli applied at various time delays initially increased the peak atrial cycle length to ~1,650 ms from a prestimulation length of 600 ms (Fig. 2A). Barium infusion increased the resting atrial cycle to 700 ms (Fig. 2B). Barium infusion markedly reduced the peak initial vagally induced increase in atrial cycle length to ~860 ms, resulting in a change of 160 ms (Fig. 2B). Despite the reduction in the initial increase in atrial cycle length, the periods of acceleration and secondary cycle length increase were of approximately the same magnitude as before the barium infusion.

An average concentration of 0.55 mM barium increased the resting atrial cycle length by 100 ms (Fig. 3A). Vagal stimulation in the presence of atrial barium perfusion attenuated the initial increase in atrial cycle length by 454 ms (Fig. 3B). Barium had no significant effect on the mean acceleratory phase or on the secondary increase in atrial interval that followed the acceleratory phase (Fig. 3, C and D, respectively).
Effects of cesium, an I\textsubscript{f} channel antagonist. In a representative experiment, the peak initial atrial cycle length increased to 2,000 ms from a prestimulation cycle length of 500 ms (Fig. 2C). This initial increase was followed by a brief period of acceleration and a subsequent secondary increase in the cycle length (\textasciitilde 70 ms). After the infusion of cesium, the response of SA nodal pacemaker cells to vagal stimulation was altered (Fig. 2D). There was no measurable change in the peak initial increase in atrial cycle length; however, the normal brief period of acceleration between the initial and secondary atrial slowing was absent as was the secondary bradycardic response. There was a gradual prolonged acceleration starting \textasciitilde 3 s after the application of the brief burst and lasting for 10–20 s. During this prolonged acceleration, the atrial cycle length was shortened by \textasciitilde 100 ms. This period of acceleration was not altered by \textbeta-\textit{adrenergic} blockade (propranolol, 1 mg/kg iv).

On average, the two lowest concentrations of cesium that we used did not significantly change the resting atrial cycle length; however, the 2.0 mM concentration...
Effects of UL-FS-49, an I_f channel antagonist. In a representative dog in the absence of UL-FS-49, vagal stimulation initially increased the atrial cycle length to \( \sim 1,250 \) ms from a prestimulation atrial cycle length of 400 ms, for a change of 850 ms. This initial increase was followed by a brief period of acceleration (325 ms) and a subsequent small but prolonged secondary increase in the cycle length (\(-50\) ms above the prestimulation level; Fig. 2E). Administration of 0.1 mg/kg of UL-FS-49 increased the prestimulation atrial cycle length from 400 to 675 ms. The initial peak in cycle length reached 1,550 ms, but the absolute increase was 875 ms (1,550 − 675 ms), a value comparable to the control value, i.e., 850 ms (compare Fig. 2, E and F). Despite the lack of a dramatic change in the initial increase in atrial cycle length, the period of relative acceleration that normally follows the initial increase in atrial cycle length was completely absent in this dog. The secondary vagally induced increases in atrial cycle length were comparable in size in the control and treated dogs.

On average, both doses of UL-FS-49, 0.05 and 0.1 mg/kg, significantly increased the mean prestimulation cycle length (Fig. 3A). The magnitude of the initial vagal prolongation was unaffected (Fig. 3B). At the higher dose of UL-FS-49, atrial acceleration was blunted (Fig. 3C). There was no significant effect of UL-FS-49 on the mean secondary increase in atrial interval (Fig. 3D).

DISCUSSION

A single vagal volley causes complex changes in sinus rhythm (2, 3, 10–12, 27–29). The changes consist of two periods of increased cardiac cycle length separated by a transient period of acceleration. To get these chronotropic changes, the vagus nerve (preganglionic) is stimulated and acetylcholine is released onto neurons in the parasympathetic ganglia and activates nicotinic receptors. The activated postganglionic neurons release acetylcholine at their nerve terminals. This acetylcholine reacts with muscarinic receptors on SA nodal membranes. We restricted delivery of two of the channel blockers, cesium and barium, by introducing the cations into the SA nodal artery. This artery supplies the right pulmonary vein fat pad, which contains cardiac parasympathetic ganglia innervating the SA node and the node itself (23). We will discuss the effects of the channel antagonists on each component of the vagal-effect curve by examining their possible actions on the transmission process.

Specificity of ionic channel antagonists. None of the channel antagonists is totally selective. However, at the concentrations that we used, one channel is affected much more than the others (2). Cesium (1–2 mM) completely blocks the I_f channel in rabbit SA nodes without affecting the delayed rectifier potassium current or the inward calcium current (6). However, in frog atrial muscle, application of 1 mM cesium partially blocks at least two potassium channels (1). In the rabbit SA node, barium concentrations in the 1-10 mM range affected several potassium channels (21). The I_{K,ACh} was not measured in the study by Shibata et al. (25). In rabbit Purkinje fibers, barium concentrations < 1 mM affected only the I_{K,ACh} (5). Concentrations above this value began to affect other currents; however, we used a concentration that was always 1 mM or less (mean concentration 0.55 mM).

The rapid initial increase in cardiac cycle length is due to hyperpolarization and a decrease in diastolic depolarization of SA nodal pacemaker cells by acetylcholine (27). Several ionic channels in the external membranes of the cardiac automatic cells mediate the negative chronotropic effect of neurally released acetylcholine. These channels include the acetylcholine-regulated potassium channels (I_{K,ACh}), channels that carry the “funny” cationic current (I_f), and channels carrying a slow inward calcium current (32). The acetylcholine-regulated potassium channels have been implicated in the initial rapid chronotropic response to vagal activity because the time course of their activation corresponds to the time course of the initial vagally induced slowing (12). The binding of acetylcholine to muscarinic receptors activates a G protein that directly binds to the potassium channels, accounting for the fast response; no additional second messenger system is interposed between the muscarinic receptor and channel activation (22). In SA cells, low concentrations of barium selectively antagonize the muscarinic I_{K,ACh} channels (2, 8). However, in ganglion cells, the nicotinic acetylcholine currents are antagonized by neither barium nor cesium (20). We report that barium blunts the initial increase in atrial cycle length in response to brief bursts of vagal stimulation. This is most likely due to its effect on SA muscarinic I_{K,ACh} channels.

DiFrancesco et al. (8) demonstrated that in isolated myocytes exposed to low concentrations of acetylcholine, the I_f channel can be regulated independently of the I_{K,ACh} channel. Inhibition of the I_f channel slows the rate of diastolic depolarization and may explain the observation that cardiac slowing can be obtained in the absence of membrane hyperpolarization when low concentrations of acetylcholine or short durations of vagal stimulation are used (25). The SA node and rat parasympathetic neurons are similar in that both exhibit an inward rectifying current (I_i) that is blocked by cesium.
but resistant to barium (31). Neither cesium nor UL-FS-49, predominantly I\textsubscript{f} channel antagonists, significantly attenuated the initial increase in atrial cycle length caused by vagal stimulation. Thus, although I\textsubscript{f} channels are involved in modulating the effect of acetylcholine on pacemaker cells (8) and on modulating intraganglionic transmission, I\textsubscript{f} channels do not significantly contribute to the initiation and magnitude of the rapid, phasic responses to vagal stimulation that we describe herein. However, cesium slows the recovery of atrial pacemaker cells from acetylcholine application, implicating the I\textsubscript{f} channel in this recovery.

As pacemaker cells spontaneously depolarize, membrane calcium permeability changes (8). This calcium permeability is altered by acetylcholine, but the relationship between these calcium permeability changes and the vagal-effect curves is unclear. In dogs, administration of the calcium-channel antagonists verapamil or nifedipine prolongs resting cardiac cycle length (14). However, the calcium-channel antagonists have no consistent effect on the initial vagal prolongation of cycle length (29).

It has been hypothesized that the “acceleratory” portion of the vagal-effect curve is due to acetylcholine hyperpolarization of the SA nodal membrane, thus activating the I\textsubscript{f} (12). In an isolated rabbit atrial preparation, 2 mM cesium completely blocks the SA nodal I\textsubscript{f} (7). We found that cesium (0.5–2 mM) and UL-FS-49 (0.05–0.1 mg/kg), I\textsubscript{f} channel antagonists, blocked the acceleratory portion of the curve. In an intact SA nodal preparation, Boyett et al. (2) reported two cases in which the acceleratory effect of vagal stimulation is abolished by cesium. They speculated that an I\textsubscript{f} channel might be involved in this part of the vagal response. In addition to blocking the I\textsubscript{f}, cesium also hyperpolarizes and then depolarizes secondary SA pacemakers (26) and may affect other depolarizing currents, at least in myocytes (24). These effects require higher concentrations of cesium than those that we used.

In isolated perfused dog atria, UL-FS-49 has a negative chronotropic effect similar to the one that we reported. Furukawa et al. (9) also reported that UL-FS-49 inhibits the effects of repetitive vagal stimulation on atrial rate. We did not see an attenuation of the vagal effect; however, it is difficult to compare the concentrations we used with those used in the study by Furukawa et al. Furukawa et al. added UL-FS-49 directly into the SA artery and stimulated it immediately thereafter. Moreover, the flow rate of the blood perfusing the artery is not given. We injected into the femoral vein and allowed 15 min for equilibration.

The mechanisms for the smaller, secondary vagally induced cardiac cycle prolongation are not known, but the duration of this secondary prolongation corresponds to the duration of an increase in extracellular potassium levels (27). The increase in extracellular potassium may activate an electrically sodium-potassium pump (27). Two of the antagonists, barium and UL-FS-49, had no appreciable effect on the small secondary vagal prolongation of atrial cycle length. The effect of one of the channel antagonists (cesium) on the secondary atrial prolongation was difficult to evaluate. A burst of vagal stimulation in the presence of cesium causes a gradual, protracted shortening of the atrial cycle that occurs during the time that one would normally observe the secondary prolongation, thus obscuring any effect. This protracted shortening of atrial cycle length is not due to activation of sympathetic fibers within the vagus nerve because it is not blocked by propranolol, a b-blocker.

Thus our results in intact dogs are consistent with the interpretation that the rapid prolongation of the cardiac cycle that is seen with vagal stimulation involves activation of I\textsubscript{f}; channels. This corroborates previous work in isolated preparations (2, 11, 12, 27). The atrial cycle acceleration that follows this initial vagal slowing is blocked by I\textsubscript{f} channel antagonists. However, because we did not actually measure membrane currents, our interpretations are based on the results of previous voltage-clamp experiments on isolated cells (2). Our results help explain a complicated cardiac response to vagal stimulation in intact dogs. Our results do not rule out the participation of other channels in the response because although the antagonists, in the concentrations that we used, each block one channel much more than others, they are not specific for only one channel.

We thank Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT) for the generous gift of UL-FS-49 and Frank Walters for excellent technical assistance.

This research was supported by National Heart, Lung, and Blood Institute Grant HL-10951 and grants from the National American Heart Association and the Northeast Ohio Heart Association.

Address for reprint requests: D. W. Wallick, Dept. of Neurobiology, Northeastern Ohio Universities College of Medicine, PO Box 95, Rootstown, OH 44272.

Received 13 December 1996; accepted in final form 19 June 1997.

REFERENCES