Thromboxane A$_2$ mimetic evokes a bradycardia mediated by stimulation of cardiac vagal afferent nerves

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Received 18 July 2001; accepted in final form 23 October 2001

Wacker, Michael J., Roya N. Tehrani, Rory L. Smoot, and James A. Orr. Thromboxane A$_2$ mimetic evokes a bradycardia mediated by stimulation of cardiac vagal afferent nerves. Am J Physiol Heart Circ Physiol 282: H482–H490, 2002. First published October 18, 2001; 10.1152/ajpheart.00624.2001.—Injections of the thromboxane A$_2$ mimetic U-46619 (10 and 20 µg) into the left atrium of anesthetized rabbits evoked decreases in heart rate (HR) and arterial blood pressure (ABP) followed by an increase in ABP. Bilateral, cervical vagotomy abolished the U-46619-induced bradycardia and attenuated the hypotension. Injections of U-46619 into the ascending aorta did not evoke the bradycardia and hypotension but did cause arterial hypertension. To further define the origin of the vagal reflex, recordings of nerve impulses were made from 11 chemosensitive cardiac vagal afferent nerves. Impulse frequency increased in all 11 fibers in response to left atrial injections of phenylbiguanide (20–30 µg) and U-46619 (5–10 µg). Onset time of nerve activity induced by U-46619 correlated with the onset time of bradycardia. We conclude that U-46619 injections into the left heart elicit decreases in HR and ABP via a vagal reflex that originates from the heart similar to the coronary chemoreflex described for other agents.

coronary chemoreflex; phenylbiguanide; prostaglandins; anesthetized rabbits

THROMBOXANE A$_2$ (TxA$_2$) is a metabolite of arachidonic acid released from plasma membranes of blood platelets (12). It has been documented that TxA$_2$ evokes platelet aggregation and smooth muscle contraction, and that the enzyme that converts prostaglandin endoperoxides into thromboxane (thromboxane synthase) is found in a wide variety of tissues (12, 25).

More recent work has shown that TxA$_2$ may have actions beyond vasoconstriction and platelet aggregation. For example, TxA$_2$ is capable of eliciting pulmonary reflexes from the lung. Shams and Scheid (34) have shown that injection of the TxA$_2$ mimetic U-46619 into the inferior vena cava elicited pulmonary hypertension and rapid shallow breathing (tachypnea). Stimulation of vagal afferent fibers by U-46619 mediated the breathing response, because vagal cooling abolished the U-46619-induced tachypnea. Further work with the TxA$_2$ mimetic has shown it stimulates unmyelinated C fibers from the hindlimb of the cat (20). Recently, Sun et al. (35) have shown that TxA$_2$, when applied to the epicardial surface in rats, stimulates cardiac nerves. These reports provide evidence that TxA$_2$ may be a significant stimulating agent of sensory nerves, thereby eliciting important cardiopulmonary reflexes.

The aim of the present study was to extend these findings and to determine whether TxA$_2$ elicits cardiac reflexes by stimulation of cardiac nerves. Stimulation of these nerves by TxA$_2$ could be especially significant during myocardial ischemia, because elevation of the levels of TxA$_2$ in the heart during myocardial ischemia has been documented by a number of laboratories (3, 15, 26). Chemical stimulation of nerves from the heart is known to elicit the coronary chemoreflex (Bezold-Jarish reflex). This reflex was originally characterized by injection of veratrum alkaloids and includes a bradycardia and arterial hypotension mediated by stimulation of cardiac vagal nerves (5, 8, 11, 13). After this earlier work with veratrum alkaloids, other endogenous chemicals, such as prostaglandins and bradykinin, have been shown to elicit these reflex changes (14, 22, 29). Because it has been reported that TxA$_2$ is released in the heart during myocardial ischemia and has been shown to stimulate chemosensitive nerves, we investigated whether this agent might stimulate cardiac vagal afferent nerves, eliciting changes in heart rate (HR) and arterial blood pressure (ABP) when injected into the left atrium.

METHODS

Animal preparation. For all experiments, male and female New Zealand White rabbits (mean weight, 4 kg) were initially tranquilized with an intramuscular injection of Rompun (2.5 mg/kg) and then anesthetized with an intramuscular injection of ketamine (35 mg/kg). Catheters were inserted into the right femoral vein and artery. Forty minutes after the ketamine injection, 3–4 ml of a solution of 2% α-chloralose (~15 mg/kg) and 10% urethane (~75 mg/kg) dissolved in a mixture of 2% borax and 98% water was given intravenously. The α-chloralose-urethane solution (2 ml) was infused at 45-min intervals to maintain anesthesia. ABP was continually monitored via the arterial catheter. The trachea was exposed, a plastic tube was inserted, and the rabbit was

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ventilated (volume 25 ml; rate 30 breaths/min). End-tidal CO₂ levels were measured to ensure proper ventilation (4–5%). The vagus nerves in the cervical region of the animal were exposed, and sutures were lightly placed around the nerves. Body temperature, which was monitored with a thermometer inserted into the rectum, was maintained near 38°C by controlling the temperature of a heating pad placed beneath the animal.

An incision was made between the third and fourth ribs on the left side of the thorax, and the ribs were spread to expose the left surface of the heart. Cotton gauze moistened with 0.9% saline was used to move the upper lobe of the left lung away from the heart. The pericardial sac was opened around an area on the left ventricle, and the heart was exposed. A catheter (polyethylene-90) was inserted into the left atrium and secured with sutures tied around the catheter and through the walls of the left atrium. This catheter was used to administer drugs into the left atrium. Four series of experiments were carried out in 45 animals. The initial preparation of the animals was the same for all series of experiments. However, for series 3, in lieu of placing a catheter in the left atrium, a catheter was inserted into the carotid artery and advanced into the left ventricle. Location of the catheter tip within the left ventricle was verified by measurement of left ventricular blood pressure. Drugs were then injected through the catheter into the left ventricle.

After a series of injections into the left ventricle, the catheter tip was then withdrawn from the ventricle approximately 2–3 cm into the aorta and its presence in the aorta was verified by measuring ABP. Drugs were then injected into the aorta.

For series 4, in addition to the initial animal preparation, the left cervical vagus nerve was isolated. The nerve was laid across a small dissecting platform, and the area was immersed in mineral oil to prevent drying. The nerve sheath was removed, and small slips of the vagus nerve were dissected and placed on bipolar recording electrodes. Nerve impulses were amplified (model P5 series; Grass), displayed on an oscilloscope (model RM661A; Tektronix), and fed into a computer with the PowerLab Chart program (ADInstruments) for recording, analysis, and discrimination of action potentials.

All experimental protocols and procedures involving the use of animals in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee (42–02).

Protocol. In series 1, the vehicle and U-46619 (0.5–10 μg) were applied to the epicardial surface of the left ventricle, U-46619, phenylbiguanide (PGB), prostacyclin (PGI₂), and prostaglandin F₂α (PGF₂α) (10 and 20 μg) were injected into the left atrium, and HR and ABP were recorded. U-46619 was administered into the opening in the pericardial sac onto the surface of the left ventricle using a micropipette. The drug was infused in 2 ml of 0.9% saline. Other concentrations of U-46619 (35 μg/ml, 10 μg/ml, and 5 μg/ml) were made by diluting the working stock solution with normal saline. PBG (Sigma), PGL₂ (Sigma), and PGF₂α (Caymon Chemical) were also prepared in the same manner. Drugs (100 μl) were injected to the epicardial surface of the heart using micropipettes. Injections into the left atrium were made via the left atrial catheter. A syringe was filled to 0.1 ml with the drug solution (for U-46619 this would yield doses equivalent to 10 μg, 3.5 μg, 1.0 μg, and 0.5 μg), and then the solution diluted with 0.2 ml of saline. The drug was infused in 2–3 s and followed by a 0.5-ml injection of saline. For purposes of comparison among the prostaglandins, the formula weights of U-46619, PGF₂α, and PGL₂ are similar, and injections into the left atrium were made with the same final volume of saline and with the same microgram quantity. Therefore, similar molar concentrations were used for the three arachidonic acid metabolites (44, 44, and 42 mM, respectively, for a 20-μg dose). A vehicle with the same ethanol concentration as the test solutions was also injected to test for the effects of the vehicle alone.

Measurements and data analysis. The right femoral artery catheter was connected to a pressure transducer to monitor systemic ABP. All data were analyzed with a commercial software package (PowerLab; ADInstruments). HR was measured from a tachometer trace of ABP. Baseline, or preinjection, measurements of HR and ABP were obtained over a 10-s time period before injection and then compared with postinjection values taken during the period of greatest change after the administration of the test drug. Data from individual experiments were averaged and the means ± SE calculated. For ease of comparison between pre- and postinjection values, selected data are reported as percent change from the baseline levels. A paired t-test was used to determine whether there were significant differences in the HR and ABP elicted by U-46619, PBG, PGL₂, PGF₂α, and the vehicle. For determination of significant differences among three means (e.g., baseline ABP, hypotension, and hypertension) a two-factor ANOVA was performed followed by a post hoc least significant difference test if significant F values were obtained. For all statistical tests, significant differences were accepted at the P < 0.05 value.
For nerve recordings, preinjection values were averaged for a 5-s period of maximal activity selected from a 30-s control period before drug injection. Postinjection values were averaged for a 5-s period of maximal activity. When more than one unit was active, individual units were counted with the aid of a window discriminator and spike-sorter program (PowerLab, ADInstruments) that discriminated different units based on both amplitude and width. Multifiber recordings were not used if different units were of similar amplitude or if there were more than 3–4 active units. Data from individual experiments were averaged, the means ± SE were calculated, a paired t-test was conducted, and a significance level of P < 0.05 was established to measure significant differences.

RESULTS

Series 1. There was no significant change in either HR or ABP after the administration of the vehicle to either the epicardial surface of the heart or left atrium (P > 0.05). Average values for HR and ABP before application of the control solution were 228 ± 6 beats/min and 64 ± 3 mmHg (n = 25).

Application of U-46619 to the epicardial surface of the left ventricle in doses up to 10 μg resulted in no significant change in HR. Although application of low doses of U-46619 (0.5 and 1.0 μg) to the epicardial surface of the left ventricle did not induce a significant change, ABP did fall slightly when higher doses were applied (5 ± 1% at the 3.5-μg dose, n = 7; P < 0.05 and 8 ± 2% at the 10-μg dose, n = 10; P < 0.05).

Injection of U-46619 into the left atrium led to significant changes in both HR and ABP. The ABP and HR response of two animals to left atrial injection of U-46619 (10 μg) is shown in Fig. 1. A noticeable arterial hypotension, as well as a decrease in HR, occurred ~12 s after the injection of U-46619 (Fig. 1A). Figure 1B illustrates the response to the same injection of U-46619 in a different animal. Again, hypotension and bradycardia occurred at ~9 s, which in this particular animal was then followed by arterial hypertension and a second period of bradycardia.

Figure 2 presents the average decrease in HR and ABP for all animals. Magnitude of the bradycardia after left atrial injection of U-46619 was variable among rabbits. Whereas some rabbits displayed a dramatic decrease in HR, there was a group of animals that showed little or no response. Of the 30 animals studied, seven exhibited less than a 5% decrease in HR at the 10- and 20-μg doses. Five of these 7 rabbits were also tested with 30 μg of U-46619 and there was still no decrease in HR.

Although magnitude of the response varied, the timing of the bradycardia was consistent among animals. Average latency of the bradycardia elicited by U-46619 was 11 ± 1 s. Bradycardia usually correlated with the onset of arterial hypotension and occurred before the arterial hypertension.

When averaging ABP values from all animals, left atrial injection of U-46619 led to biphasic changes in ABP (Fig. 2B). At both the 10- and 20-μg doses, ABP fell, initially followed by an increase in ABP. However, responses were variable among animals (Fig. 1), i.e., depending on the individual, U-46619 injections sometimes led to only arterial hypotension or only hypertension. In most cases, the hypotension was transient, lasting <30 s. However, in nine rabbits at the 20-μg dose (two at the 10-μg dose), hypotension persisted for a period of up to 10 min. In three rabbits at the 20-μg dose, a dramatic arterial hypotension developed that persisted for a period of more than 15 min until blood pressure was almost zero, arrhythmias developed, and the rabbit eventually died.

Series 2. Six of the rabbits from series 1 used for the left atrial injections were selected for vagotomy studies in series 2. Figure 3 presents the percent change in HR evoked by U-46619 injection before and after bilateral cervical vagotomy. Vagotomy eliminated the decrease in HR.
in HR after U-46619 injection. In the animals that displayed hypotension (3 of 6 and 4 of 6 at the 10- and 20-μg doses, respectively), the average percent decrease in ABP before vagotomy was 18 ± 5% for the 10-μg dose and 23 ± 4% for the 20-μg dose. After vagotomy, hypotension was not eliminated in all animals but was reduced to an average percent decrease in ABP of 10 ± 6% for both doses. In one animal at the 20-μg dose, a dramatic hypotension lasted 16 min. After vagotomy, this prolonged hypotension did not occur. The hypotension produced by epicardial application of U-46619 was also eliminated by vagotomy.

After injection of U-46619 into the left atrium, arrhythmias sometimes developed. An arrhythmic event was observed in one-fourth of the rabbits after the 10-μg dose and in over one-third of the rabbits at the 20-μg dose. Onset time of these arrhythmias was usually from 40 to 120 s after injection.

Injection of 20 μg of PBG into the left atrium elicited a decrease in HR of 6% (n = 6; P < 0.05 comparing pre- and postvagotomy). HR changes began 4–8 s after PBG was administered. No bradycardia was observed after vagotomy. Injections of 20 μg of PBG elicited an average decrease in ABP of 23 ± 4%. Vagotomy eliminated the hypotension in three of five rabbits.

Injecting PGI2 (20 μg) did not cause a significant decrease in HR (n = 6; P > 0.05 comparing pre- and postvagotomy). Only one animal displayed a significant change in HR (15% decrease). The onset time of that bradycardia was 10 s, and it was eliminated after vagotomy. Injections of PGI2 elicited variable responses in ABP, ranging from no response to hypertension or hypotension before and after vagotomy.

Injection of PGF2α (20 μg) into the left atrium did not elicit a decrease in HR < 5% in any of the experiments (n = 6). A dose of 120 μg was tested and only one of six animals showed a decrease in HR > 5%. The decrease in HR was 14% in this one experiment and the onset time was 7 s. All of the experiments with PGF2α displayed a biphasic change in ABP, with a period of decrease and a period of increase sometime after the injection. At the 20-μg dose there was a decrease of 10 ± 3% and an increase of 8 ± 1%. At the 120-μg dose there was a decrease in only one of six animals (8%), but all six showed an increase with an average of 23 ± 2%.

Series 3. To better define whether the reflex changes in HR originated from the heart, the site of the U-46619 injection was varied. Figure 4 displays the percent change in HR induced by U-46619 (10 μg) injections into the left ventricle and into the aorta. In addition to the HR changes that occurred over a similar time course as that measured for the left atrial injections (labeled prehypertension) being measured, HR was also measured during the period of hypertension induced by U-46619 injection. Left ventricular injections lead to decreases in HR at both periods, similar to left atrial injections (see Fig. 1B). The first bradycardia occurred with an onset time of 10 ± 1 s and occurred before an increase in ABP. The second bradycardia occurred at the peak of hypertension (onset time of 23 ± 4 s). Injections into the aorta did not lead to bradycardia at the early time period but elicited a larger decrease in HR during the increase in ABP (onset time of 20 ± 1 s). All four of the rabbits displayed an increase in ABP with injections into the left ventricle and aorta. Aortic injections led to a larger average
increase in ABP (49 ± 14%) compared with left ventricular injections (34 ± 16%). With injections into the left ventricle, three of the rabbits displayed a hypotension (12 ± 7% decrease) preceding the hypertension. No hypotension was observed after injection of U-46619 into the aorta.

Series 4. To define the afferent mechanism for the reflex change in HR, 11 chemosensitive cardiac afferent nerve fibers were tested. None of these fibers had a pattern of firing synchronized with the heartbeat; rather, all firing patterns were irregular. Figure 5 presents two examples of multiunit nerve recordings from two different rabbits. The unit with the large amplitude in each recording was discriminated by the action potential amplitude and width. Each of these units was stimulated in response to PBG and U-46619. Figure 6 presents the average changes in impulse frequency to all 11 fibers after the injection of PBG, U-46619, and the vehicle. The action potential frequency of all 11 fibers increased in response to probing of the heart and after injections of PBG and U-46619. Nine fibers responded to probing of the left ventricle, whereas two fibers responded to probing of the left atrium. The average onset time to PBG and U-46619 stimulation was 7 ± 1 and 11 ± 1 s, respectively. Response to PBG usually consisted of a short, strong burst with the duration of the response lasting between 5 and 20 s. Response to U-46619 usually involved a longer duration of stimulation, lasting between 5 and 80 s.

**DISCUSSION**

**U-46619-induced bradycardia.** Injections of U-46619 into the left atrium or left ventricle of the anesthetized rabbit elicited two periods of bradycardia. Initial bradycardia (average onset time of 11 s) occurred coincidentally with arterial hypotension and before an increase in ABP. A second, more latent bradycardia was usually correlated with the increase in ABP (Fig. 1). Because the second bradycardia likely involves the baroreceptor reflex, this study focused on the initial bradycardia that occurred coincidentally with the hypotension.

Decrease in HR after left atrial injections of U-46619 was variable among rabbits. Although the majority of rabbits displayed a decrease in HR that ranged from 5 to 20%, another group of rabbits did not respond to U-46619, and a third group exhibited a strong response. Failure of some animals to respond to U-46619 (nonresponders) has been reported by other laboratories. In a study with isolated blood vessels from rabbits, Buzzard et al. (2) reported that 25% of the pulmonary or aortic segments did not respond positively to TxA2 mimetics. These nonresponders had a significant decrease in the number of vascular TxA2 receptors. Interestingly, there appears to be no strong correlation...
between the magnitude of the bradycardia (cardiac response) and the strength of the arterial hypertension (vascular peripheral response). It was noticed, however, that the occurrence of strong bradycardia correlated positively with bradycardia that accompanied the increase in ABP, indicating perhaps a difference in the level of vagal control in some animals.

In preliminary experiments, U-46619 was injected in the left atrium in small doses (0.5, 1.0, and 3.5 μg). A slight bradycardia and more apparent hypotension were observed at the 3.5-μg dose. It is therefore concluded that the threshold dose of U-46619 needed to elicit reflex bradycardia via left atrial injection was near 3.5 μg.

U-46619 (up to 10 μg) did not elicit changes in HR and only small decreases in ABP when applied to the epicardial surface of the heart. Two other studies have also tested responses to TxA₂ on the epicardial surface of the heart. Pickar (31), in work on anesthetized cats, administered U-46619 into the pericardial sac of the heart. He previously reported that stimulation of visceral afferent nerves inhibits somatic motor responses including the knee-jerk reflex. Using U-46619 as a tool to stimulate visceral afferent nerves, Pickar showed that the drug inhibited the knee-jerk reflex when injected intravenously but had no effect when administered into the pericardial sac of the heart. Pickar’s data are in agreement with the present data, because there was little cardiovascular response to epicardial U-46619 application.

However, results by Sun et al. (35) show an effect when applying TxA₂ to the epicardial surface of the heart in rats. They found that applications of TxA₂ stimulated cardiac vagal afferent nerves at a level greater than equimolar concentrations of PGI₂, PGE₂, and PGF₂α. It is possible that epicardial applications of U-46619 during our experiment stimulated afferent nerves, but the strength of the stimulation was not enough to elicit reflex changes in HR. This may be possible, because Sun et al. (35) also reported no significant cardiovascular changes after application of TxA₂. Because different doses and methodologies were used in the studies by Sun et al. (35), Pickar (31), and our work, it is difficult to directly compare the results. However, these results suggest that there may be species differences with regards to the location of TxA₂-sensitive afferents.

Absence of strong cardiovascular responses to epicardial application of U-46619 is not unique to this drug. Other investigators (1, 9) have found that intrapericardial applications of PBG up to 400 μg in rabbits had no effect. However, left atrial injections of PBG (50–200 μg) produced significant decreases in HR and ABP that the authors concluded to be due to stimulation of myocardial afferents, because the reflex changes were eliminated by intrapericardial procaine (9). It is possible that in rabbits, as well as other species, the fibers stimulated by U-46619 and PBG are located deeper in the myocardium and are not accessible from the epicardial surface.

Vagal reflex. Decrease in HR evoked by left atrial injection of U-46619 was a reflex, because the response was eliminated after bilateral cervical vagotomy. Comparisons were made with other substances (PGF₂α, PGI₂, and PBG) known to stimulate cardiac nerves and elicit vagally mediated reflex changes in HR (1, 9, 13, 14, 22). PGF₂α and PGI₂ have formula weights similar to U-46619, and therefore, similar molar concentrations resulted from a 20 μg injection of each. Although PGF₂α and PGI₂ led to much weaker HR changes than U-46619, the time course of the bradycardia was similar to U-46619, and the decrease was eliminated by vagotomy. Koss and Nakano (22) found similar results, i.e., injections of PGF₂α in the left atrium of cats (1–4 μg/kg) resulted in hypotension and bradycardia with an average onset time of 8.8 ± 2.3 s. Injections of PBG also produced a hypotension and small vagally mediated bradycardia with a shorter onset time (4–8 s). Reflex decreases in HR and ABP elicited by U-46619 are comparable to the coronary chemoreflex described by other authors for various chemical agents (5, 8, 9, 11, 13, 14, 22, 29).

After vagotomy, arterial hypotension produced by U-46619 was reduced, but not completely eliminated. Experiments with PBG provided results similar to those with U-46619; i.e., the hypotension induced by this agent was not always eliminated after cervical vagotomy. Evans et al. (9) have also reported that left atrial injections of PBG produced a hypotension not completely eliminated by bilateral cervical vagotomy. It is possible that sensory nerves that travel through the spinal cord or other pathways besides the main trunk of the vagus nerve contribute to arterial hypotension. Other investigators have reported depressor responses to epicardial application of bradykinin attributed to stimulation of sympathetic afferent nerves (10, 30). Our experiments suggest that extravagal mechanisms may be partially responsible for the observed depressor responses.

Hypertension produced by U-46619 injection is likely due to the vasoconstricting actions of the TxA₂ mimetic. In addition to the documented evidence of TxA₂...
as a vasoconstrictor (4, 12, 25), there are two lines of evidence from our experiments that support this conclusion. First, epicardial applications of U-46619 did not elicit a hypertension (series 1), and second, when U-46619 was injected into the aorta, hypertension was larger than the response after injections into the left atrium or left ventricle (series 3).

Cardiac origin. Although our results correlate with the coronary chemoreflex elicited by other chemicals, it could be argued that left atrial injections led to systemic distribution of the drug, and therefore, the responses that we observed were brought about by a noncardiac effect. Reflex bradycardia may be due to stimulation of afferent nerves originating from organs such as the lungs (18, 31, 34) or higher brain centers (7). To determine whether stimulation of noncardiac nerves contributed to the responses, injections of U-46619 into the heart were compared with injections of U-46619 distal to the heart (ascending aorta). When U-46619 was injected into the left ventricle, a response similar to left atrial injection was observed. Bradycardia occurred coincidentally with arterial hypotension and before any increases in ABP. Another bradycardia typically occurred at the peak of hypertension. However, injections into the aorta resulted in no early bradycardia or hypotension, but a larger hypertension, and a larger bradycardia occurred during this hypertension. We conclude, therefore, that the initial bradycardia is due to a reflex originating from the heart and that bradycardia during hypertension was due to activating the baroreceptor reflex. We also propose that the hypotension, although not completely eliminated by vagotomy, is due to a reflex of cardiac origin. Two lines of evidence support this hypothesis: first, there was no hypotension after injection into the aorta; and second, there was a small hypotension that occurred on application to the epicardial surface.

Stimulation of cardiac vagal afferent nerves. To examine the origin of the reflex further, afferent recordings of impulse frequency were made from chemosensitive cardiac vagal afferent nerves. Afferent units were chosen based on their irregular discharge, low baseline frequency (characteristic of chemosensitive units), and positive response to propping of the heart (5, 6, 11, 19). Units that discharged in a rhythmic fashion, coincidentally with contraction of the heart, were not studied, because such units are more likely to be mechanoreceptors. All units responded to propping of the left ventricle or left atrium and to injection of PBG and U-46619. This corresponds with the fact that other researchers have found that the left ventricle and left atrium contain vagal fibers that respond to chemical stimuli (5, 6, 8, 11, 13, 14, 19, 22). Although the strength of response of these chemosensitive units to PBG varied, the response was similar i.e., the units almost always had a short, rapid increase in action potential frequency, a short time of onset (average of 7 s), and a short duration of stimulation (5–20 s). U-46619 stimulated these same units with an overall larger average increase in firing, but the onset time was more delayed (average of 11 s), and the duration of stimulation was longer (5–80 s). The average times of onset for PBG and U-46619 correspond with the times of onset measured for bradycardias recorded in series 1 and 2. Therefore, stimulation of cardiac afferent nerves by PBG and U-46619 is likely responsible for the observed initial reflex change in HR. It is not likely that U-46619 stimulated these nerves via an increase in ventricular blood pressure, because the time of onset of neural stimulation usually occurred during the arterial hypotension and before the rise in ABP. Likewise, it is not likely that the volume of fluid injected stimulated the afferent units, because the vehicle was injected with the same volume and did not elicit a significant increase in impulse frequency or change in HR. It could also be argued that because U-46619 induced arrhythmias in some animals, the arrhythmias are responsible for the increase in cardiac afferent activity. However, we used smaller doses in the nerve-recording experiment (5–10 μg) that rarely produced arrhythmias. Higher doses of U-46619 sometimes produced arrhythmias, but the average onset time of the arrhythmias was 78 s and therefore began much after the onset of stimulation of cardiac nerves by U-46619.

The exact mechanism of stimulation of afferent nerves by U-46619 is unknown. The endogenous receptors for other substances that elicit the coronary chemoreflex (PBG, PGI2, and bradykinin) have been localized to the vagus nerve and nodose ganglia (vagus nerve cell body) (16, 23, 24). Ligation of the vagus nerve was used in the study with PGI2 to verify that receptors were transported from the nodose ganglia cells to the peripheral terminals (24). However, the TxA2 receptor has not yet been localized to sensory afferent nerves, but it is possible that U-46619 elicited bradycardia by stimulation of vagal afferent nerves via a direct receptor-mediated event. It is also possible that the reflex changes evoked by U-46619 are due to the release of another substance that then stimulates the vagus nerve to elicit changes. TxA2 appears to be a significant stimulator of sensory nerves, and the precise mechanism of stimulation awaits further investigation.

Significance. It has previously been shown that the TxA2 mimetic stimulates pulmonary vagal afferents (18) and group III and IV fibers from the hindlimb (20). We now report that the TxA2 mimetic stimulates cardiac vagal afferent units and elicits reflex changes in cardiovascular function. We believe these experiments provide further support for the importance of TxA2 in stimulation of sensory fibers to elicit reflexes. Specifically, TxA2 may be a strong stimulator of vagal afferent nerves, because injections both in the heart and, as reported previously, to the lungs (20, 31, 34) led to reflex changes mediated by the vagus.

Stimulation of cardiac vagal afferent nerves by TxA2 can lead to decreases in HR and ABP similar to the response previously described for the coronary chemoreflex (5, 8, 9, 11, 13, 14, 22, 29) and similar to the reflex hypotension and bradycardia observed during coronary ischemia (17, 36, 39, 40). It has been suggested that these depressor reflexes that occur during
ischemia may provide a protective function in reducing the ischemic injury to the heart by decreasing HR and ABP and thereby reducing the workload of the heart (28). This reflex may be even more significant in those cases where there is an ischemic event on the inferior, posterior, portion of the heart (32, 36, 39, 40).

Evidence suggests TxA2 may play a significant role in stimulation of cardiovascular function during coronary ischemia. Recently, Ustinova and Shultz (37) have shown that pretreatment with indomethacin (an inhibitor of prostaglandin and TxA2 formation) prevented activation of chemosensitive cardiac vagal afferent nerves at the beginning of left anterior descending artery occlusion in the rat. This would indicate a significant role for arachidonic acid metabolites during ischemia in stimulating cardiac nerves. Evidence from our results as well as from Sun et al. (35) suggests that TxA2 may be a more potent arachidonic acid metabolite than other prostaglandins in stimulating cardiac vagal afferent nerves. Because it has been shown that TxA2 is released during myocardial ischemia (3, 15, 26) and we have shown that TxA2 stimulates cardiac reflexes and stimulates cardiac nerves, there is strong evidence to suggest that TxA2 stimulates nerves and mediates reflex cardiovascular changes during coronary ischemia.

It is also possible that stimulation of the vagus nerve may play a role in the arrhythmias generated during myocardial ischemia. Reports (27, 38) have shown that stimulation of the vagus nerve may be protective against severe arrhythmias produced during myocardial ischemia. However, it has also been reported that excessive vagal activity and vagally mediated bradycardia may also induce arrhythmias during myocardial ischemia (21, 33). It is possible that during myocardial ischemia, TxA2 may alter the susceptibility of the heart to arrhythmias via stimulation of cardiac nerves or the generation of bradycardia.

In summary, the TxA2 mimic stimulates cardiac vagal afferent fibers to elicit reflex changes in HR and ABP. When released during myocardial ischemia, TxA2 may elicit reflexes that could alter cardiac function and possibly, arrhythmias.

We thank John Tyburski and Dr. Joel Pickar for useful scientific discussions that contributed to the completion of this project. This work was supported by Grant-in-Aid 0051148Z from the American Heart Association, Heartland Affiliate.

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