Role of serotonin in thromboxane A2-induced coronary chemoreflex

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Wacker, M. J., H. L. Wilhelm, S. E. Gomez, E. Floor, and J. A. Orr. Role of serotonin in thromboxane A2-induced coronary chemoreflex. Am J Physiol Heart Circ Physiol 284: H867–H875, 2003. First published October 31, 2002; 10.1152/ajpheart.00617.2002.—We reported previously that the thromboxane A2 (TxA2) mimetic U-46619 stimulates cardiac vagal afferent nerves, eliciting a reflex decrease in heart rate (HR) and arterial blood pressure (ABP). The present experiments were designed to test the hypothesis that TxA2 evokes these changes via the release of serotonin [5-hydroxytryptamine (5-HT)] and activation of the 5-HT3 receptor. Injections of the 5-HT3 antagonist tropisetron (1 mg of 3-tropanyl-indole-3-carboxylate or ICS-205-930) attenuated the decreases in HR and ABP induced by left atrial injections of U-46619 (20 µg). Tropisetron administration also eliminated the U-46619-induced increase in impulse frequency in a majority of cardiac, vagal afferent units tested. Measurement of serum 5-HT levels after U-46619 injection in those rabbits that displayed a significant HR change following injection of U-46619. These results indicate that although other factors may also contribute to these reflex responses, the release of 5-HT and stimulation of the 5-HT3 receptor plays a significant role in coronary reflexes induced by TxA2.

U-46619; tropisetron; phelybiguanide; vagal afferent units; anesthetized rabbit

We have previously reported that left atrial injections of a thromboxane A2 (TxA2) mimetic, U-46619, decreases heart rate (HR) and arterial blood pressure (ABP) in the anesthetized rabbit (45). These reflex responses were mediated by stimulation of cardiac, vagal afferent nerves. U-46619 stimulation of these afferent nerves may be mediated directly by the action of TxA2 on cardiac, vagal afferent nerves or indirectly via the release of other substances.

It is well established that TxA2 receptors are present on platelets, and stimulation of these receptors by TxA2 receptor agonists activates platelets leading to the release of serotonin [5-hydroxytryptamine (5-HT)] (2, 20, 23, 28, 34, 42). Because 5-HT has been shown to stimulate the vagus nerve and evoke the coronary chemoreflex via stimulation of 5-HT3 receptors (1, 10, 11, 13, 26, 33), there is a strong rationale to suspect that 5-HT release and stimulation of the 5-HT3 receptor mediates at least part of the TxA2 responses that we have previously reported. We therefore chose to investigate the hypothesis that injection of U-46619 stimulates these reflex effects via the release of 5-HT and the subsequent actions of 5-HT on cardiac, vagal afferent neurons.

Methods

Animal preparation. For all experiments, male New Zealand White rabbits (mean weight = 4 kg) were initially tranquilized with an intramuscular injection of xylazine (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. Thirty to fifty 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 were given intravenously. Two milliliters of the α-chloralose-urethane solution were infused at ~40-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 ventilated (volume 25 ml; rate 30 breaths/min). End-tidal CO2 levels were monitored to ensure proper ventilation.

Four series of experiments were carried out in a total of 59 animals. The initial preparation of the animal was the same for all series of experiments. 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 lung away from the heart. The pericardial sac was opened around an area on the left ventricle and the heart exposed. A catheter (polyethylene-90) was inserted into the left atrium and secured with sutures that were tied around the catheter and through the walls of the left atrium. This catheter was then used to administer drugs into the left atrium.

For series 3, in addition to the initial animal preparation described above, 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 of the nerve. The nerve sheath of the vagus was removed, and small slips of the vagus nerve were dissected and placed on bipolar recording electrodes. Recording electrodes were connected to a high-impedence probe (model RPS 107B, Grass Instruments; Quincy, MA), and the signal was amplified (Grass P511 amplifier). These signals were displayed on an oscilla-
scope (Tektronix RM561A; Beaverton, OR) with low and high filters set at 10 Hz and 1 kHz, respectively, and input was fed into the PowerLab Chart program with the Spike Sorter extension (ADInstruments), which was installed on a G4 Macintosh for recording, analysis, and discrimination of action potentials.

For series 4, an additional catheter was inserted into the left femoral artery and advanced 3–4 inches. Just before the U-46619 injection and at selected times after injection of U-46619, blood was sampled from the arterial catheter. For several reasons, we chose to measure 5-HT levels in blood sampled from the femoral artery. First, 5-HT concentrations in the femoral artery should reflect 5-HT concentration in blood destined for the coronary circulation. Second, the femoral artery is much more accessible than either the coronary artery or coronary sinus, thereby simplifying surgical procedures and reducing trauma to the animal.

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 (IACUC no. 42-02).

Drug µg per g). TxA2 degrades to the inactive metabolite TxB2 under physiological conditions (half-life ~30 s). Therefore, the stable TxA2 mimetic U-46619 (Caymon Chemical) was used to stimulate TxA2 receptors (7). A stock solution was made by dissolving 10 mg of U-46619 in 1 ml of 100% ethanol. A working stock solution of 100 µg/ml was made by removing 0.1 ml from the stock solution and adding 9.9 ml of 0.9% saline. The 5-HT3 receptor agonist phenylbiguanide (PBG) (Sigma) was prepared by making a concentration of 1 mg/ml deionized water. 5-HT was purchased as 5-HT anide (PBG) (Sigma) was prepared by making a concentration of the drug solution and then diluted with 0.1 ml of saline. The drug was infused in 3–10 min. After injection of U-46619, PBG was given and then 1 mg of the 5-HT3 receptor antagonist tropisetron was injected into the left atrium. After injection of the 5-HT3 antagonist, 15 min elapsed before the injections of PBG and U-46619 were repeated. In a different group of rabbits (series 1b), the effect of the vehicle for tropisetron was tested by administering PBG and U-46619 before the vehicle and again 15 min following injection of the vehicle.

In series 2, HR and ABP were recorded following a protocol that was similar to series 1a except that 5-HT was injected instead of U-46619. PBG (400 µg) was injected and followed by 5-HT (100 µg). These injections were repeated 15 min following administration of tropisetron (1 mg).

In series 3, slips of nerve fibers were teased from the cervical vagus nerve and tested for identification of cardiac chemosensitive units. Each fiber was cut, and the peripheral end of the nerve was laid on recording electrodes to measure only afferent signals. Afferent units were chosen based on their pattern of discharge. Typically, nerve units that respond to chemical stimuli have a low random frequency of firing under baseline conditions (8, 9, 19, 27). The origin of the afferent unit was determined by lightly probing the heart with a cotton tip (8, 9, 19, 27). PBG (20–30 µg) was then injected into the left atrium to verify the chemosensitive nature of the unit. If an afferent unit responded to both probing of the heart and injection of PBG, then the response of the unit to U-46619 (5–10 µg) was tested. Injections of PBG and U-46619 were repeated before injection of tropisetron to verify the reproducibility of responses from repeated injections. Five minutes were allowed between each drug injection. Tropisetron (1 mg) was then injected into the left atrium, and 15 min were allowed before subsequent injection of PBG and U-46619. Low doses of U-46619 and PBG were selected for these experiments to reduce nerve sensitization and to reduce dramatic ABP changes. The vehicle for U-46619 was administered after U-46619 and PBG injections to ensure that neither the volume of fluid injected nor the solvent for the drug stimulated the receptor unit.

In series 4, blood was sampled 1 min before injection of U-46619 blood and then sampled again between 15 and 45 s after injection of U-46619. Blood was also sampled before and after injection of the vehicle for U-46619 during the same time course in a separate group of rabbits. The following protocol was modified from Wright and Angus (46). Blood (2 ml) was slowly withdrawn with a 10-ml syringe and then emptied into a test tube containing 25 mg ascorbic acid (Sigma). Blood was allowed to clot for 45 min and then centrifuged at 5,000 rpm (3,000 g). The serum was then removed and frozen at −20°C for storage until measurement with HPLC.

Measurements and data analysis. The arterial catheter was connected to a pressure transducer to monitor systemic ABP. All data were collected 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 taken over a 10-s time period before injection and then compared with postinjection values taken during the period of greatest change following administration of the test drug. To examine the mechanism of the U-46619-induced decrease in HR, only animals that responded to U-46619 with a decrease in HR (baseline) were included in the data collection. 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 the percent change from the baseline levels. A paired t-test was used to determine whether there were significant differences in the HR and ABP elicited by U-46619, PBG, 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 test (least significant difference) 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 from the 30-s period just 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), which 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 three to four 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 chosen to establish significant differences.
Measurements of serum 5-HT levels were made by using HPLC analysis with electrochemical detection. The analysis was performed by using a 100 × 3.2-mm phase II ODS column (BioAnalytical Systems; West Lafayette, IN) in 10% acetonitrile-90% 0.15 M chloroacetate buffer, pH 3.0, containing 0.7 mM EDTA and 0.86 mM octane sulfonate (32) with electrochemical detection at 0.6 V, using an LC-4C Amperometric Detector (BioAnalytical Systems). The 5-HT concentration (in pmol/l) was calculated with reference to HPLC injections of 10 and 20 pmol of 5-HT standards.

RESULTS

Series 1. Figure 1 presents the ABP and HR response following injection of PBG and U-46619 into an animal before tropisetron (A and C, respectively) and after treatment with tropisetron (1.0 mg iv) (B and D, respectively). Typically, left atrial injections of PBG elicited a decrease in ABP and HR with an average onset equal to 6 ± 1 s following the injection. U-46619 injections usually elicited a decrease in ABP followed by an increase in ABP. The decrease in HR usually occurred during the decrease in ABP. The average time of onset of this bradycardia was 9 s. However, some animals also displayed another more latent decrease in HR, which occurred during the elevated ABP. Because this HR change was likely due to the baroreceptor reflex, only the decrease in HR that occurred before the increase in ABP and during the decrease in ABP was measured. As shown in Fig. 1, the HR change induced by U-46619 was eliminated following injection of tropisetron, whereas the blood pressure change was attenuated but not eliminated. The average changes in HR and ABP after injection of PBG and U-46619 in series 1a are shown in Fig. 2 (n = 8). In previous studies, it has been documented that HR does not decrease in all rabbits following injections of U-46619 (45). Because the goal of this study was to determine the mechanism of the U-46619-induced decrease in HR, only those rabbits that exhibited a HR change >5% (i.e., “responders”) were included in the analysis of data. In series 1a and 1b using U-46619, 15 of 21 rabbits exhibited a HR decrease >5%. PBG was also tested in the six “nonresponders.” The average decrease in HR following injection of PBG was 8 ± 1% in the nonresponders compared with an average change of 17 ± 3% in the responders. Neither HR nor ABP changed significantly (P > 0.05) following injection of the vehicle for U-46619.

To verify that repeated injections of PBG and U-46619 would yield consistent changes in HR and ABP, injections of PBG and U-46619 were administered to a series of animals before and after the tropisetron vehicle (n = 7). The results for series 1b are displayed in Table 1.

Series 2. In this series, the HR and ABP responses to 5-HT and PBG were tested. Similar to U-46619, 5-HT elicited a decrease in ABP followed by an increase in ABP. There was also a decrease in HR that occurred during the decrease in ABP with an average time of onset of 5 ± 1 s. The average time of onset for PBG was 6 ± 1 s. The average changes in HR and ABP elicited...
Fig. 2. Average changes in HR (A) and mean ABP (MABP) (B) induced by left atrial injections of 400 μg PBG and 20 μg U-46619 before and after tropisetron (Trop, n = 8). Note that PBG usually only caused a decrease in ABP, whereas U-46619 elicited a decrease in ABP followed by an increase in ABP. *Significant difference at \( P < 0.05 \) comparing baseline and postinjection values; + significant difference at \( P < 0.05 \) comparing hypotension and hypertension values.

Fig. 3. Average changes in HR (A) and MABP (B) induced by left atrial injections of 400 μg PBG and 100 μg 5-hydroxytryptamine (5-HT) before and after injection of tropisetron (n = 9). Note that 5-HT shows that PBG usually caused a decrease in ABP, whereas 5-HT elicited a decrease in ABP followed by an increase in ABP. *Significant difference at \( P < 0.05 \) comparing baseline and postinjection values; + significant difference at \( P < 0.05 \) comparing hypotension and hypertension values.

Table 1. HR and ABP changes before and after vehicle

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<td>211 ± 17</td>
<td>203 ± 17</td>
<td>223 ± 15</td>
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<td>MABP</td>
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<td>69 ± 4</td>
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Values are means ± SE. HR, heart rate; MABP, mean arterial blood pressure; PBG, phenylbiguanide; U4, U-46619. *Significant difference at \( P < 0.05 \) compared with baseline.

Four units from our previous study (45) as well as eight new units. Figure 4 displays a multiunit recording from the previous study as well as a new unit. Both nerve recordings in Fig. 4 display multiunit activity, but the unit with the large amplitude action potential in each recording was easily discriminated from other action potentials and analyzed. Both units responded to probing of the left ventricle and to injections of both PBG and U-46619 (Fig. 4, A and C, respectively). The units did not show an increase in impulse frequency to either PBG or U-46619 after injection of tropisetron (Fig. 4, B and D, respectively).

The average changes in impulse frequency in all the units tested (Fig. 5) support the responses that are shown for individual animals. Injections of PBG and U-46619 were repeated before injection of the 5-HT₃ antagonist to verify that repeated injections of PBG and U-46619 would yield consistent results. The aver-
average time of onset for the increase in activity was 6 ± 1 s for PBG and 9 ± 1 s for U-46619. After tropisetron injection, there was no statistically significant increase in average afferent unit impulse frequency to PBG and U-46619. Eight of twelve afferent units displayed no increase in impulse frequency after U-46619 injection in the presence of tropisetron; however, four units still elicited an increase in impulse frequency. Three of these four units displayed a percent increase in impulse frequency to U-46619 injection equal to that before tropisetron injection.

Series 4. Serum 5-HT levels before and after injection of saline or U-46619 are shown in Fig. 6. Rabbits were grouped based on treatment and the presence or absence of a response to the treatment. A total of 15 animals were used with the rabbits either being given injections of the U-46619 vehicle (n = 4) or U-46619 (n = 11). Rabbits given U-46619 were grouped according to their HR response to the drug (i.e., responders vs. nonresponders). The responders (n = 6) displayed a significant and consistent increase in 5-HT levels, whereas the nonresponders (n = 5) elicited variable changes in 5-HT levels that were not statistically significant (P > 0.05). The average HR for the responders was 242 ± 23 beats/min before U-46619 injection and 214 ± 20 beats/min after injection. The average HR for the nonresponders was 225 ± 15 beats/min before U-46619 and 219 ± 15 beats/min after U-46619.

DISCUSSION

Coronary chemoreflex. Previous work (45) from our lab has shown that left atrial injections of the TxA2 mimetic U-46619 stimulate cardiac afferent units from the vagus nerve and elicit a decrease in HR and ABP. The purpose of this study was to determine the role of...
the 5-HT$_3$ receptor in the observed U-46619-induced responses. We hypothesized that injections of U-46619 would induce the release of 5-HT, which then stimulates the 5-HT$_3$ receptor and mediates the neural and cardiovascular responses elicited by the TxA$_2$ mimetic.

Similar to our previous work, left atrial injections of U-46619 led to a decrease in HR and a biphasic change in ABP. After tropisetron administration, the decrease in HR was significantly reduced, whereas the decrease in ABP was attenuated. The more latent increase in ABP that is induced by U-46619 is likely due to systemic vasoconstriction. This increase in ABP persisted following 5-HT$_3$ receptor blockade. Because it could be argued that repeated injections of the TxA$_2$ mimetic U-46619 could induce tachyphylaxis resulting in a decrease in the HR and ABP response, repeated U-46619 injections were carried out. We observed that the decreases in HR and ABP were the same following repeated injections of the TxA$_2$ mimetic. We conclude from these data that a significant portion of the U-46619-induced coronary chemoreflex that we have observed in these rabbits is mediated by the stimulation of the 5-HT$_3$ receptor.

We also compared U-46619 responses to those observed following left atrial injection of 5-HT. The results with 5-HT mimic the responses to U-46619, further supporting our hypothesis that TxA$_2$ may induce reflex changes via the release of 5-HT. Injections of 5-HT elicited a decrease in HR and a biphasic change in ABP, and these average decreases in HR and ABP were reduced after tropisetron administration. The increase in ABP was not reduced but rather augmented after tropisetron administration. The increase in ABP is hypothesized to be due to systemic vasoconstriction either elicited directly by 5-HT or via the release of TxA$_2$ or another vasoactive agent.

It is interesting to note that in these reflex studies there was a variation in the degree of HR change in these rabbits, similar to our previous studies (45). In the current report (series 1a and 1b), 6 of 21 rabbits did not display a decrease in HR >5% in response to injection of the TxA$_2$ mimetic. In comparison, these same six nonresponder rabbits also did not respond with as large an average decrease in HR to injections of PBG as the responders. We also observed that 2 of 11 rabbits did not evoke a decrease in HR >5% to injection of 5-HT. These nonresponders may not show a strong response to these agents for a variety of reasons, including a lower number of 5-HT or TxA$_2$ receptors, differential dampening of the neural response in some animals due to the anesthetic drugs, or variability of the vagal response among animals. There is also a strong possibility that in some animals U-46619 and 5-HT injections did not evoke a large release of 5-HT from platelets or other sources, and therefore the response was dampened.

The presence of nonresponder rabbits has been reported by other groups. Buzzard et al. (5) have shown that 25% of pulmonary or aortic segments in rabbits did not contract in response to TxA$_2$ mimetics. They found that the nonresponders had a significant decrease in the number of vascular TxA$_2$ receptors. However, in these nonresponders there was no decrease in TxA$_2$ receptor binding sites in platelets and no change in platelet aggregation to U-46619. In a study more similar to ours, Wright and Angus (46) observed that injections of the 5-HT$_1$-like agonist, 5-carboxamidotryptamine, in the rabbit elicited the Bezold-Jarisch-like reflex, and this effect was blocked with a 5-HT$_3$ receptor antagonist. They concluded that 5-carboxamidotryptamine elicited the release of 5-HT from platelets and subsequently evoked the reflex via stimulation of 5-HT$_3$ receptors. They also observed a longer delay in the Bezold-Jarisch-like response to 5-carboxamidotryptamine compared with the response observed to injections of 5-HT or PBG, which is similar to our findings with U-46619. Similarly, they observed that 30% of the rabbits studied did not elicit a reflex response to 5-carboxamidotryptamine. They attributed these observations to the fact that 5-carboxamidotryptamine did not elicit a strong enough release of 5-HT. These results are similar to our findings in that the responders had a significant increase in serum 5-HT levels whereas the nonresponders did not. When the findings of Buzzard et al. (5) and Wright and Angus (46) are compared with our results, it is possible that the TxA$_2$ receptor density in platelets in nonresponders are similar to responders, but the 5-HT levels contained in platelets is either lower or the signal transduction mechanism for release of 5-HT is less effective.

**Afferent nerve fiber recordings.** To further investigate the mechanism for the tropisetron-induced reduction in the coronary chemoreflex, recordings were made from cardiac afferent units within the vagus nerve. These units were determined to originate from either the left atrium or left ventricle based on increases in impulse frequency in response to mechanical probing of the heart (8, 9, 19, 27). All of these units responded to PBG. Before administration of tropisetron, PBG and U-46619 were given twice to ensure that repeated stimulation of an afferent unit would cause reproducible increases in impulse frequency (Fig. 5). Of the 12 nerve fibers tested, all responded with an increase in impulse frequency to U-46619, although the magnitude of the response varied. After tropisetron administration in 8 of 12 fibers, there was no increase in impulse frequency in response to U-46619, although the magnitude of the response varied. After tropisetron administration in 8 of 12 fibers, there was no increase in impulse frequency in response to U-46619. Three units still displayed a percent increase in frequency similar to that before tropisetron administration. It is interesting to note that these three fibers did not respond as strongly as other fibers to PBG before tropisetron. It is possible that these three units were not as sensitive to 5-HT and were more responsive to other stimuli, such as direct stimulation by TxA$_2$ or stimulation due to the release of some other substance. It is not likely that these units were stimulated by mechanical changes in the heart, because the onset of the response occurred before the increase in ABP and with the same time course as the other fibers.

Tropisetron, the antagonist utilized in our study, has been widely used as a 5-HT$_3$ receptor-blocking agent.
The dose of tropisetron that was used in this study has been used in similar protocols to block the 5-HT₃ receptor (15, 16, 30). It has also been reported that tropisetron is a weak antagonist for the 5-HT₄ receptor (14, 40). Therefore, it is possible that some of the actions of tropisetron in our study may result from blockade of the 5-HT₄ receptor. However, Freeman et al. (14) report that the affinity of tropisetron for the 5-HT₃ receptor is ~1,000:1 over other receptors. Furthermore, the actions of 5-HT in eliciting the coronary chemoreflex would most likely be due to stimulation of the 5-HT₃ receptor. It has been well documented that 5-HT₃ receptor antagonists inhibit the depolarizing actions of 5-HT on isolated vagus nerve and nodose preparations (1, 11, 26) and eliminate the 5-HT-evoked coronary chemoreflex (10, 13, 33). The presence of 5-HT₃ receptors on sensory neurons has been established (21, 48), and radioactively labeled tropisetron has been used to localize 5-HT₃ receptors in both rabbit vagal and nodose preparations (25). Therefore, we hypothesize that the actions of tropisetron in this study are due to 5-HT₃ receptor antagonism.

PBG was used as a control in all of these studies because it is an accepted 5-HT₃ receptor agonist, is documented to stimulate cardiac nerves, and elicits decreases in HR and ABP (3, 12). As shown in the present study, PBG elicited a decrease in HR and ABP in all the series tested, and these cardiovascular changes were effectively eliminated after administration of tropisetron. Therefore, we are confident that we effectively blocked the 5-HT₃ receptor during these studies.

**5-HT release.** Even though previous authors have reported that TxA₂ can elicit the release of 5-HT from platelets (20, 28, 42), we chose to verify that there was a rise in 5-HT in our preparations using the chosen dose of U-46619. Comparing blood samples taken from the femoral artery before and after U-46619 injection, the responders displayed a significant elevation of 5-HT in the serum, whereas the average 5-HT concentration did not significantly change in the nonresponders. The concentrations of serum 5-HT that we measured were consistent with reports from other authors (29, 37, 46). Rabbits contain a larger quantity of 5-HT in their platelets than other species including humans (37), and therefore it is possible that the release of 5-HT from sources such as platelets in rabbits is much larger than other species, and therefore the effects of 5-HT could mask other direct or indirect actions of TxA₂. In the rat, Sun et al. (41) have shown that TxA₂ applied to the epicardial surface (where there is less likely to be 5-HT release by platelets) stimulates cardiac vagal afferent nerves in the anesthetized rat to a level comparable to prostaglandin (PG)₁₂ and PGE₂.

**Other factors contributing to cardiovascular responses.** We conclude that U-46619 induces a significant portion of these cardiovascular and neural changes via the 5-HT₃ receptor in the anesthetized rabbit. However, after 5-HT₃ receptor blockade, because there was a residual decrease in HR and ABP and an increase in impulse frequency in a small group of units tested, it is possible that other factors may also play a role in the U-46619-induced coronary chemoreflex.

Even though it is generally accepted that the coronary chemoreflex evoked by 5-HT is due to stimulation of the 5-HT₃ receptor, it is possible that other 5-HT receptors may also be involved. Specifically, in the isolated rat vagus nerve it has been shown that there is a residual depolarization induced by 5-HT after 5-HT₃ receptor antagonism by granisetron or odansetron, which is then eliminated following blockade of the 5-HT₁ receptor (4, 6, 39). Therefore, the 5-HT₄ receptor may play a minor role in the 5-HT-induced responses. Further work will have to be conducted to more fully investigate the role of the 5-HT₄ receptor in these responses. It has also been suggested that the 5-HT₂ receptor may play a role in 5-HT responses (47). However, other research has demonstrated a lack of a role of 5-HT₂ receptors in stimulation of the vagus nerve by 5-HT (4, 39, 46). Previous work from our lab supported no involvement of the 5-HT₂ receptor in U-46619-evoked reflexes because the 5-HT₂ receptor antagonist ketanserin did not block reflex responses evoked by TxA₂ in the anesthetized cat (36).

It is also possible that U-46619-induced depolarization of the vagus nerve is mediated or possibly modulated by the TxA₂ receptor. It has been shown that members of the prostaglandin family PG₁₂, PGE₂, and TxA₂ induce increases in firing of sensory neurons (22, 41, 45), and the receptors for PG₁₂ and PGE₂ (IP and EP, respectively) have been localized to sensory neurons (31). Although the TxA₂ receptor (TP) has yet to be localized to sensory neurons, preliminary work from our lab has shown that TP mRNA is present in the cell bodies of cultured sensory neurons (43).

The possibility for direct (nonneuronal) actions as well as other factors cannot be ruled out. It is not likely that U-46619, 5-HT, or other released substances would elicit a direct effect on the heart affecting HR because previous work showed that the HR decrease was eliminated after vagotomy (45). However, the decrease in ABP was not completely eliminated after vagotomy, and it is possible that U-46619 or 5-HT evoked the release of other mediators from platelets or elsewhere and elicited vasodilation. It is also possible that pulmonary vasoconstriction induced by U-46619 or 5-HT would alter the cardiac output and transiently lower ABP. Further work will be needed to investigate these other factors; however, from our studies in the anesthetized rabbit, it can be concluded that the 5-HT₃ receptor does play a significant role in the U-46619 coronary chemoreflex.

**Significance.** It is concluded from these data that U-46619 can induce changes in afferent unit activity, HR, and ABP via release of 5-HT and subsequent stimulation of the 5-HT₃ receptor. It has been demonstrated that TxA₂ elicits platelet aggregation and can induce the release of 5-HT from platelets (2, 20, 23, 28, 34, 42). Therefore, the mechanism of this stimulation is likely the release of 5-HT from activated platelets. This
may provide another significant role for TxA₂ in that release of TxA₂ during tissue trauma may trigger a cascade of events involving platelets and 5-HT that induce significant cardiovascular changes.

The role of platelets and 5-HT in stimulating afferent nerves during myocardial ischemia has been documented. Fu and Longhurst (17) have shown that platelet-rich plasma plus collagen (activated platelets) can stimulate cardiac afferents, and decreasing the circulating platelet number during myocardial ischemia can attenuate the ischemia-induced increase in impulse activity in cardiac spinal afferents. They have found that they can inhibit this platelet-induced response with tropisetron (30).

Likewise, thromboxane may also be important in stimulating nerves during ischemia. It has been shown that TxA₂ is released during myocardial ischemia (24, 35) and that activation of cardiac vagal afferent nerves by left anterior descending artery occlusion in the rat was attenuated by pretreatment with indomethacin (an inhibitor of prostaglandin and TxA₂ formation) (44). Recent work has now shown that TxA₂ also stimulates ischemically sensitive cardiac afferent nerves that travel through the spinal cord in the anesthetized cat and may activate these afferents during ischemia (18).

It is possible that the actions of TxA₂, 5-HT, and platelets may all be intertwined during myocardial ischemia and stimulate nerves via a cascade of events, which involve activation and amplification of platelets, TxA₂, 5-HT, or other factors. Specifically in this study, we have reported that TxA₂ may play a significant role in stimulating cardiovascular reflexes by stimulating the release of 5-HT and subsequent stimulation of the 5-HT₃ receptor.

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