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Thromboxane A2-induced arrhythmias in the anesthetized rabbit

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Thromboxane A2 (TxA2) mimetic U-46619. Arrhythmias were primarily of ventricular origin, dose dependent in frequency, and TxA2 receptor mediated. The response was receptor specific since arrhythmias were absent after pretreatment with a specific TxA2 receptor antagonist (SQ-29548) and did not occur in response to another prostaglandin, PGF2α. Alterations in coronary blood flow were unlikely the cause of these arrhythmias because coronary blood flow (as measured with fluorescent microspheres) was unchanged after U-46619, and there were no observable changes in the ECG-ST segment. In addition, arrhythmias did not occur after administration of another vasoconstrictor (phenylephrine). The potential involvement of autonomic cardiac efferent nerves in these arrhythmias was also investigated because TxA2 has been shown to stimulate peripheral autonomic cardiac efferent nerves in these arrhythmias. Pretreatment of animals with the β-adrenergic receptor antagonist propranolol did not reduce the frequency of these arrhythmias. Pretreatment with atropine or bilateral vagotomy resulted in an increased frequency of arrhythmias, suggesting that parasympathetic nerves may actually inhibit the arrhythmogenic activity of TxA2. These experiments demonstrate that left atrial injection of U-46619 elicits arrhythmias via a mechanism independent of a significant reduction in coronary blood flow or activation of the autonomic nervous system. It is possible that TxA2 may have a direct effect on the electrical activity of the heart in vivo, which provides significant implications for cardiac events where TxA2 is increased, e.g., after myocardial ischemia or administration of cyclooxygenase-2 inhibitors.

autonomic nervous system; coronary blood flow; prostaglandin F2α; phenylephrine; SQ-29548; U-46619

BECAUSE THROMBOXANE A2 (TxA2) IS RELEASED DURING PLATELET ACTIVATION AND TISSUE TRAUMA, THE IMPORTANCE OF DEFINING THE INVOLVEMENT OF THIS CYCLOOXYGENASE (COX)-CATALYZED METABOLITE IN THE DEVELOPMENT OF ARRYTHMIAS THAT ACCOMPANY MYOCARDIAL ISCHEMIA IS APPARENT. ADDING ADDITIONAL SIGNIFICANCE TO THIS STUDY IS THE RECENT ATTENTION ON THE POTENTIAL ADVERSE CARDIOVASCULAR EFFECTS AFTER INHIBITION OF THE COX-2 ENZYME. THE POSSIBLE INVOLVEMENT OF TxA2 IN ARRHYTHMIAS AFTER INHIBITION OF COX-2 COULD HAVE IMPORTANT IMPLICATIONS ON THE USE OF NONSTERoidal ANTI-INFLAMmATORY DRUGS THAT ALTER LEVELS OF TxA2.

The importance of TxA2 in generating arrhythmias during myocardial ischemia has been previously documented. Coker et al. (7) in a study with anesthetized greyhounds reported elevated levels of thromboxane B2 (TxB2, a stable metabolite of TxA2) in venous blood sampled from an ischemic region of the heart after ligation of the left anterior descending coronary artery. In this previous study, there was a correlation between the magnitude of increases in TxB2 and the frequency of arrhythmias generated after the ligation. In subsequent investigations, it was reported that pretreatment with the TxA2 receptor antagonists AH-23848 and UK-38485 reduced the number of arrhythmias that were observed after left anterior coronary artery occlusion (5, 6). Additional work by other researchers has documented similar findings in that thromboxane synthase inhibitors or TxA2 receptor antagonists reduce or prevent arrhythmias induced by coronary artery occlusion (44, 51).

Previous work from our laboratory has focused on the ability of TxA2 to stimulate peripheral nerves (20, 21). Recently, we have demonstrated that U-46619 stimulates cardiac vagal nerves and elicits vagally mediated decreases in heart rate (HR) and mean arterial blood pressure (MAP) (48, 50). In the course of these previous experiments we have noted that arrhythmias occurred in some animals after left atrial injections of the TxA2 mimetic U-46619. It is noteworthy that the arrhythmias that we observed after left atrial injection of U-46619 occurred in the absence of coronary artery ligation.

Because TxA2 is a known vasoconstrictor (16, 28), cardiac injections of TxA2 could lead to coronary vasoconstriction, ischemia, and subsequent arrhythmias. Likewise, previous reports that TxA2 stimulates cardiac nerves leads one to hypothesize that the autonomic nervous system could also participate in the genesis of these TxA2-induced arrhythmias. We therefore designed a series of experiments to test the hypothesis that reductions in coronary blood flow and stimulation of autonomic nerves contribute to the development of arrhythmias after left atrial injections of U-46619. As reported below, the data fail to support either of these hypotheses, opening the

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possibility that TxA₂ may have direct effects on the electrical activity of the heart.

METHODS

Drug preparation. TxA₂ degrades to the inactive metabolite TxB₂ under physiological conditions (half-life, ~30 s). Therefore, the stable TxA₂ mimetic U-46619 (Cayman Chemical; Ann Arbor, MI) was used to stimulate the TxA₂ receptor (8). Solutions of U-46619 were made as previously described (48, 50). PGF₂α, (Cayman Chemical) was prepared in the same manner as U-46619. For purposes of comparison among the prostaglandins, the formula weight of U-46619 and PGF₂α are similar, and therefore the molar concentrations are comparable. SQ-29548 (Cayman Chemical) was prepared by dissolving 10 mg in 2 ml of ethanol and 13 ml of PBS. All of the following compounds were purchased from Sigma (St. Louis, MO) and prepared in water at the following concentrations: atropine, 1 mg/ml; acetylcholine, 0.1 mg/ml; isoproterenol, 0.5 mg/ml; and propranolol, 10 mg/ml.

Animal preparation. 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). For all experimental procedures, male New Zealand White rabbits (mean weight = 4 kg) were initially tranquilized with an intramuscular injection of xylazine, followed by an intramuscular injection of ketamine, and anesthesia was maintained with injections of α-chloralose-urethane. Maintenance of anesthesia and surgical preparation of the animal (including inserting catheters into the femoral artery and vein, mechanical ventilation, opening of the chest, and inserting a catheter into the left atrium) have been described in previous reports from this laboratory (48, 50).

Protocol. Some procedures were the same in all experiments. HR, arterial blood pressure (ABP), and a lead II ECG were recorded continuously throughout all experiments. All drugs were injected into the left atrium, and a period of 5 min was allowed for recovery between each injection. In cases where arrhythmias were still present just before injection and then compared with postinjection values just before injection and then compared with postinjection values. Baseline, or preinjection, measurements of HR and ABP were measured during a 10-s time period before any injection of the drug, the recovery period was extended until the electrical activity of the heart returned to a normal rhythm. In cases where U-46619 was administered to the animal, the injection was made in triplicate with incremental increases in dose (10, 20, 30 µg). Injections into the left atrium were made via the left atrial catheter and a syringe filled with the appropriate amount of drug solution and then diluted with 0.1 ml of saline. This drug solution was infused over a period of 3–4 s, followed by a flush with 0.5 ml of saline.

For measurement of blood flow, four different fluorescent colors of NuFlow microspheres [Interactive Medical Technologies (IMT), Irvine, CA] were injected into the left atrium at different time points of the experiment. For each injection, a syringe was filled with 0.4 ml of microspheres (2.5 million spheres/ml) and further filled to 1.0 ml with saline and injected over a 30-s time period and then flushed with 0.5 ml saline. During each microsphere injection a reference blood sample was collected (at a rate of 1.03 ml/min) from the left femoral artery 30 s before the injection and until 2 min after injection of the microspheres. Once the experiment was complete, the animal was killed, and the ventricles were excised. The tissue was weighed and used to IMT for measurement of the fluorescence. Validation of the NuFlow Microsphere and IMT service is provided by the Fluorescent Microsphere Resource Center website (http://fmrc.pulmc.washington.edu/).

The overall study consisted of nine series of experiments that were carried out in 87 animals. The nine series of experiments were as follows. In series I, a single dose response to U-46619 was performed in each animal. In series II, two dose responses, separated by a 25-min recovery period, were carried out in each animal. In series III, the TxA₂ receptor antagonist SQ-29548 (10 mg), was injected followed by injection of U-46619 (30 µg) within 1–2 min after administration of the receptor antagonist. In series IV, responses to PGF₂α (10, 20, 120 µg) were tested in each animal. In series V, different fluorescent microspheres were injected at the following time points: before any U-46619 injections (baseline), before the 30-µg dose of U-46619 (~3 min), during the peak of systemic hypertension induced by the 30-µg dose of U-46619 (~30 s after injection), and 5 min after the 30-µg injection. In series VI, phenylephrine (10 and 25 µg) was injected into the left atrium. In series VII, propranolol (6 mg) was injected before injection of U-46619. To verify blockade of the β-adrenergic receptors in this series, isoproterenol (2 µg) was administered before and after propranolol injection, as well as after U-46619 injections. In series VIII, atropine (2 mg) was injected before injection of U-46619. To verify blockade of the acetylcholine muscarinic receptor, acetylcholine (50 µg) was administered before and after atropine as well as after the U-46619 injections. Finally, in series IX, a bilateral cervical vagotomy was performed before injection of U-46619. A summary of the entire series of experiments is provided (see Table 2).

Typically, transient changes in ABP occurred in these animals after U-46619 injection. However, in some animals (n = 17) the injection of the larger U-46619 dose was followed by a prolonged hypotension that persisted for up to 5 min or longer. ST segment changes were usually observed subsequent to the prolonged, severe hypotension, and some animals died after this severe arterial hypotension. In cases where significant periods of arterial hypotension followed U-46619 injections or ST segment changes occurred, the data were not included in the averages in each series because the severe changes in blood pressure and ST segment would complicate the interpretation of the mechanism responsible for these arrhythmias.

Measurements and data analysis. The right femoral artery catheter was connected to a pressure transducer to measure systemic ABP. All data were collected with a commercial software package (Powerlab, ADInstruments; Colorado Springs, CO). HR was measured from tachometer traces of ABP and ECG. Baseline, or preinjection, measurements of HR and ABP were measured during a 10-s time period just before injection and then compared with postinjection values taken during the period of greatest change after administration of the test drug (typically 5–30 s after the injection). All data are presented as means ± SD.

ECG leads were attached so as to record a lead II ECG. ECGs were analyzed manually by visual inspection of the records. Arrhythmias were counted as events where there was an abnormal QRS complex or premature QRS complex. For the experiment in which doses of U-46619 were repeated in the same animal, a two-way ANOVA was performed to determine statistical difference. For the autonomic nervous system study, a repeated-measures ANOVA followed by Tukey’s honestly significant difference post hoc test was performed to determine if treatment groups (no treatment; propranolol; atropine; vagotomy) differed statistically.

For blood flow calculations, the number of microspheres that become lodged in the precapillary vessels of the tissue area is proportional to the regional blood flow of that tissue. Regional blood flow can be calculated by the following equation: regional blood flow = no. of tissue spheres/[tissue weight in g] × (no. of reference spheres/reference flow]. Animals were first divided into groups with those that displayed arrhythmias and those that did not display arrhythmias after U-46619 injections. A series of separate unequal t-tests was performed comparing blood flow between these two groups for each time period. Because no difference was observed, data were combined (see Table 2) and a two-way ANOVA was used to determine if there was a statistical difference for coronary blood flow at different time points. For all statistical tests, significant differences were accepted at the P < 0.05 value. All statistical analysis was performed using the Microsoft Excel Data Analysis package or SPSS software (Chicago, IL).
RESULTS

**U-46619 injection elicits arrhythmias.** Left atrial injection of U-46619 at doses of 10, 20, and 30 μg elicited arrhythmias as measured with a lead II ECG. In general, U-46619 induced premature QRS complexes with abnormal morphology. The QRS complexes with abnormal morphology sometimes occurred as isolated events but more commonly were observed as bigeminy, salvos, or as a series of events (Figs. 1 and 2). In these arrhythmias, the sinus rhythm was usually unchanged from baseline as can be observed in Fig. 1. As illustrated in Fig. 1, the P waves are documented and appear at a regular frequency. However, the P-R interval becomes shortened, and QRS complexes occur earlier and are wider than the preinjection or baseline values. Note that a normal QRS complex (with normal P-R interval) does appear infrequently and most likely occurs when the ventricle is not refractory from premature depolarization. To quantify the ECG changes, P-P interval, P-R interval, and QRS duration were measured in four animals. As shown in Table 1, the average P-P interval stayed constant, and the P-R interval decreased, and the QRS duration was widened in the abnormal beats compared with the preinjection recordings.

In some cases, U-46619 also induced arrhythmias with normal QRS morphology that occurred earlier than the baseline QRS complex. Sometimes the sinus rhythm was accelerated, and in other cases it was not. However, these were usually isolated events (1 or 2 events) that occurred infrequently (in < 25% of the animals that exhibited arrhythmias).

**Dose response.** Increasing doses of U-46619 injected into the left atrium produced a corresponding increase in the frequency of arrhythmias (n = 28) (see Table 2 and Fig. 3 for the responses). The number of arrhythmias at a given dose ranged from 1 to 897, and the time of onset ranged from 8 to 140 s. The number of animals that displayed more than one arrhythmia at the 10-, 20-, and 30-μg doses was 7/28, 9/28, and 16/28, respectively.

Changes in MABP after injection of U-46619 were similar to responses that have been previously reported (48, 50). These changes involved a transient hypotension (approximate duration of 10 s) that was followed by a transient hypertension (approximate duration of 10–30 s) (Table 2).

**Repeated injections of U-46619.** The number of arrhythmias that was elicited by a repeated series of U-46619 injections was fewer than the number observed during the initial dose response (n = 8) (Table 2). The number of animals with more than one arrhythmia at the 10-, 20-, and 30-μg dose after the first injection of U-46619 were 3/8, 3/8, and 5/8, whereas 2/8, 2/8, and 2/8 developed arrhythmias after the second round of injections, respectively. The number of arrhythmias at a given dose in the first round ranged from 1 to 897 and from 1 to 171 in the second round, while the time of onset ranged from 6 to 140 s in the first dose response compared with 15 to 120 s in the second dose response.

SQ-29548. ECG and cardiovascular responses to U-46619 (30 μg) were measured after pretreatment with the TxA2 receptor antagonist SQ-29548. Injection of U-46619 elicited no arrhythmias after receptor blockade, and increases in MABP response to U-46619 were significantly reduced compared with the nontreatment group (Table 2).

**PGF2α.** ECG and cardiovascular responses to injections of PGF2α (10, 20, and 120 μg) were also measured in a separate group of rabbits (n = 6). No arrhythmias were observed after PGF2α injection at any dose. PGF2α induced changes in MABP similar to that of U-46619. The MABP before and after administration of the 10-μg and 20-μg dose of PGF2α was 63 ± 9, 59 ± 11, and 68 ± 11 mmHg (10 μg) and 71 ± 14, 64 ± 15, and 79 ± 17 mmHg (20 μg) for the preinjection, hypotension, and hypertensive events, respectively. The response of the highest dose (120 μg) is presented in Table 2.

**Coronary blood flow.** In this series, the number of animals that developed arrhythmias at the 10, 20, and 30 μg dose were 1/11, 5/11, and 6/11. The response to 30 μg of U-46619 is included in Table 2. Statistical analysis revealed that there was no significant difference in blood flow between those animals that displayed arrhythmias and those that did not (baseline P = 0.39, −3 min P = 0.32, +30 s P = 0.81, +5 min P = 0.75). Coronary blood flow data from all animals (Table 3) revealed no differences in blood flow at the different time points. The baseline blood flow rates are comparable to previously reported measurements in rabbits (15, 36).

**Phenylephrine.** Injection of phenylephrine into the left atrium of these animals elicited a strong increase in MABP. The onset of the hypertension occurred within 5–10 s with a duration of approximately 30–60 s. Phenylephrine at a 10-μg dose increased MABP from 65 ± 17 to 94 ± 17 mmHg and from 66 ± 17 to 109 ± 15 mmHg at the 25-μg dose (Table 2). No arrhythmias were observed after the 10-μg dose of phenylephrine. At the 25-μg dose, only one isolated arrhythmia was

![Fig. 1. Lead II ECG tracings before (baseline) and after injection of 30 μg of the thromboxane A2 (TxA2) mimetic U-46619. Sinoatrial nodal depolarization (as indicated by dashed lines) illustrates that the rate is not altered from baseline levels. However, the QRS complex becomes accelerated and widened compared with the baseline typical of an accelerated idioventricular rhythm. Normal QRS complexes (A, B, and C) were observed during the arrhythmias, which indicates that the conducting system of the ventricle is functional and can conduct a normal impulse when the ventricle is not refractory. P, P waves.](http://ajpheart.physiology.org/DownloadedFrom/http://ajpheart.physiology.org/10.1152/ajpheart.00356.2006)
observed in one animal at the peak of hypertension (onset time of 10 s).

**Autonomic nervous system.** Responses to the TXA2 mimetic after pretreatment with propranolol, atropine, or bilateral vagotomy are summarized in Table 2. Pretreatment of animals with propranolol had no appreciable effect on the number of arrhythmias elicited by U-46619. The number of arrhythmias at a given dose ranged from 26 to 685, and the time of onset ranged from 20 to 154 s.

For verification of the efficacy of propranolol, a β-adrenergic agonist (isoproterenol) was given before and after propranolol injection as well as after the U-46619 injections. HR changes induced by isoproterenol injection were monitored before and after propranolol and at the end of the experiment. The average HR before the initial isoproterenol injection was 208 ± 27 beats/min and increased to 264 ± 32 beats/min after injection. After propranolol injection, the HR values before and after isoproterenol injection were 190 ± 26 and 192 ± 24 beats/min, respectively. After the last U-46619 injection, the HR values before and after isoproterenol injection were 184 ± 28 and 188 ± 28 beats/min, respectively.

Blockade of the muscarinic receptor resulted in a general increase in the number of arrhythmias compared with the nontreatment group. The number of arrhythmias ranged from 1 to 1,047, and the time of onset ranged from 11 to 180 s. Similar to the propranolol series, to ensure that the dose of atropine that was used was effectively blocking the acetylcholine muscarinic receptors, acetylcholine was given before and after atropine treatment, as well as at the end of the experiment. The average HR before the initial acetylcholine injection was 219 ± 20 beats/min and decreased to 98 ± 47 beats/min after acetylcholine injection. After atropine injection, the HR values before and after acetylcholine injection were 222 ± 15 and 220 ± 15 beats/min, respectively. Acetylcholine was also given at the end of the experiment, and HR before injection was 242 ± 20 beats/min and 238 ± 20 beats/min after injection.

When U-46619 was injected after a bilateral cervical vagotomy (n = 10), the number of arrhythmias increased compared with the results from nonvagotomized animals (series I). The number of arrhythmias at a given dose ranged from 4 to 3,136, and the time of onset ranged from 8 to 130 s.

**DISCUSSION**

**U-46619 elicits arrhythmias.** We have observed that abnormal cardiac rhythms occur after injection of the TXA2 mimetic U-46619 into the left atrium of anesthetized rabbits. Other researchers have reported that TXA2 plays a major role in inducing arrhythmias during coronary artery occlusion; however, our model has demonstrated that injection of the TXA2 mimetic, in the absence of manual occlusion of a coronary artery, also evokes arrhythmias.

Although electrical mapping of the heart to precisely localize the origin of these arrhythmias was not carried out in these experiments, some general observations of the arrhythmias can be made from an analysis of the ECG recordings. The observed arrhythmias typically consisted of abnormal, premature QRS complexes.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Arrhythmias</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-P interval, s</td>
<td>0.2838 ± 0.0199</td>
</tr>
<tr>
<td>P-R interval, s</td>
<td>0.0629 ± 0.0062</td>
</tr>
<tr>
<td>QRS duration, s</td>
<td>0.0596 ± 0.0079</td>
</tr>
</tbody>
</table>

Averages (±SD) of selected measurements of a lead II ECG in 4 animals that developed arrhythmias. Measurements were taken during 20 normal beats before 30 μg U-46619 injection and 20 abnormal beats that developed after injection.
Coronary blood flow values were calculated using fluorescent microspheres that were injected into the left atrium at various time points. Baseline represents the start of the experiment before U-46619 injections. The other times of injections are indicated as relative to the U-46619 (30 μg) injection. There was no statistical difference between groups (P = 0.90).

Table 3. Coronary blood flow values

<table>
<thead>
<tr>
<th>Coronary blood flow, ml/min/kg</th>
<th>Baseline</th>
<th>−3 min</th>
<th>+30 s</th>
<th>+5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.00 ±0.97</td>
<td>2.17 ±0.75</td>
<td>2.09 ±0.46</td>
<td>2.22 ±0.87</td>
</tr>
</tbody>
</table>

Values are means ± SD; doses are in μg and mean arterial blood pressure (MABP) values are in mmHg. MABP was measured before injection of the drug (baseline), at the largest increase in MABP, and just before the onset of arrhythmias. The number of animals that displayed more than one arrhythmia after injection of the drug (frequency) and the average number of arrhythmias were also counted for each of the 9 series of experiments. U4, U-46619.

Table 2. Data summary

<table>
<thead>
<tr>
<th>Series</th>
<th>Experiment</th>
<th>Dose</th>
<th>Baseline</th>
<th>MABP</th>
<th>Arrhythmias</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Increase</td>
<td>At arrhythmia</td>
<td>Frequency</td>
</tr>
<tr>
<td>I</td>
<td>U4</td>
<td>30</td>
<td>68 ±14</td>
<td>89 ±19 (31%)</td>
<td>76 ±12</td>
</tr>
<tr>
<td>II</td>
<td>U4 repeat</td>
<td>30</td>
<td>70 ±9</td>
<td>92 ±19 (31%)</td>
<td>73 ±5</td>
</tr>
<tr>
<td>III</td>
<td>U4 + SQ-29548</td>
<td>30</td>
<td>76 ±13</td>
<td>81 ±10 (7%)</td>
<td>65 ±9</td>
</tr>
<tr>
<td>IV</td>
<td>U4-Blood flow</td>
<td>120</td>
<td>65 ±9</td>
<td>79 ±11 (23%)</td>
<td>72 ±6</td>
</tr>
<tr>
<td>V</td>
<td>Phenylephrine</td>
<td>25</td>
<td>66 ±17</td>
<td>109 ±15 (65%)</td>
<td>13 ±12</td>
</tr>
<tr>
<td>VI</td>
<td>U4 + propranolol</td>
<td>30</td>
<td>70 ±13</td>
<td>85 ±13 (21%)</td>
<td>75 ±12</td>
</tr>
<tr>
<td>VII</td>
<td>U4 + atropine</td>
<td>30</td>
<td>73 ±11</td>
<td>94 ±12 (29%)</td>
<td>77 ±15</td>
</tr>
<tr>
<td>VIII</td>
<td>U4 + vagotomy</td>
<td>30</td>
<td>68 ±12</td>
<td>98 ±22 (44%)</td>
<td>68 ±15</td>
</tr>
</tbody>
</table>

Values are means ± SD; doses are in μg and mean arterial blood pressure (MABP) values are in mmHg. MABP was measured before injection of the drug (baseline), at the largest increase in MABP, and just before the onset of arrhythmias. The number of animals that displayed more than one arrhythmia after injection of the drug (frequency) and the average number of arrhythmias were also counted for each of the 9 series of experiments. U4, U-46619.

It was observed that increasing doses of U-46619 caused an increase in the number of animals exhibiting arrhythmias as well as an increase in the average frequency of arrhythmias per animal. These data suggest that the doses were still in a physiologically relevant range because there was an increased response with the increasing dosage. While there was a dose response observed, not all animals displayed arrhythmias after U-46619 injection. The variability of the rabbit TxA2 receptor response observed, not all animals displayed arrhythmias after U-46619 likely triggered an area of depolarization in the ventricle before the conduction of the normal impulse through the ventricle. The ventricular rhythm was therefore increased over the inherent rate of ventricular depolarization. A normal QRS complex does appear intermittently at those times when the atrioventricular node is not refractory from a premature event. The appearance of a normal QRS complex indicates that the normal conducting system of the ventricle is still functional and capable of conducting a normal impulse.

Repeated injections. Before we attempted to manipulate various components of the autonomic nervous system or alter other experimental variables, we investigated whether repeated dose responses with U-46619 would elicit comparable results. If two successive dose responses yielded identical data, then a baseline dose-response test could have been compared with a second dose response after administration of a selected treatment (e.g., propranolol). Such an experimental design would have conserved animals and reduced the variability between pre- and posttreatment data. However, when two dose-response tests were repeated in the same animal, the second series of injections did not induce as strong an arrhythmogenic response as the first series of injections. The mechanism of this desensitization awaits future investigations and could be important in reducing the arrhythmogenic effects of TxA2. Although there was no statistical difference between the groups, the strong tendency for a substantial loss of response led us to design subsequent experiments whereby only one dose response was carried out in each animal.

SQ-29548 and PGF2α. While U-46619 has been shown to be an effective and selective agonist of the TxA2 receptor, several series of experiments were carried out to determine if the arrhythmogenic response to U-46619 was specific to the TxA2 receptor or could be mediated by other agents with similar actions to U-46619. In one series of experiments, animals were pretreated with the highly selective TxA2 receptor antagonist, SQ-29548. No arrhythmias were observed after 30 μg of U-46619, supporting the specificity of the receptor response.
In a second series, the prostaglandin PGF$_{2\alpha}$ was also tested in a separate group of animals for comparison to U-46619. PGF$_{2\alpha}$ was selected as a comparative agent for several reasons. Any promiscuity of U-46619 is likely to be at the PGF$_{2\alpha}$ receptor (1), and therefore it is possible that the arrhythmic actions of U-46619 may induce these arrhythmias via stimulation of the PGF$_{2\alpha}$ receptor. Poncic et al. (33), in a study of isolated ventricular cardiomyocytes, concluded that the PGF$_{2\alpha}$ receptor mediated increases in inositol 1,4,5-trisphosphate (IP$_3$) and protein synthesis induced by prostaglandins and U-46619. Also, previous research has shown that PGF$_{2\alpha}$ has been shown to induce arrhythmias. In a study of cultured neonatal rat cardiac myocytes, various eicosanoids were added to the medium of the cells. PGF$_{2\alpha}$ and U-46619 were more potent than other prostaglandins (PGD$_2$ and PGE$_2$) in inducing fast beating frequencies (tachyarrhythmias) and abnormal beating frequencies in these spontaneously beating myocytes (24). Our findings demonstrated that while PGF$_{2\alpha}$ elicited similar alterations in MABP, PGF$_{2\alpha}$ even at doses $\sim$4 times that of U-46619 did not elicit any arrhythmias. It is unlikely, therefore, that the PGF$_{2\alpha}$ receptor plays a significant role in the U-46619-mediated arrhythmias.

**Coronary blood flow.** TXA$_2$ elicits vasoconstriction (16, 28), and therefore it is logical to hypothesize that injections of U-46619 into the left atrium may have induced arrhythmias via constriction of coronary arteries. A reduction in blood flow and subsequent reduction in oxygen could disrupt the normal ion concentrations around or within myocytes and thus trigger arrhythmias. However, the lack of statistical differences in the coronary blood flow values before and after U-46619 injection indicates that the observed transient changes in MABP do not significantly alter the coronary blood flow. Despite the fact that TXA$_2$ is a vasoconstrictor, a likely reason for the absence of a significant decrease in coronary blood flow is the large increase in MABP after distribution of the drug in the systemic circulation. The increase in MABP may have counteracted the increase in coronary vascular resistance, leading to no measurable change in coronary blood flow.

This lack of apparent ischemia after U-46619 injections in our model is also supported by additional evidence. First, we observed no ST segment changes in the reported animals before the onset of arrhythmias. In addition to monitoring electrical activity with lead II measurements, we also recorded a 12-lead ECG in two animals that displayed arrhythmias. No ST segment changes were observed in any of the leads before the start of the arrhythmias. Second, injections of phenylephrine failed to produce an arrhythmic response. When responses to U-46619 were compared with this $\alpha$-adrenergic agonist and vasoconstricting agent (47, 53, 54), injections of phenylephrine led to larger increases in systemic MABP than injections of U-46619 and yet only elicited a single arrhythmia in one animal. This arrhythmia occurred at the peak of hypertension and was probably directly induced by the increase in pressure in the aorta or heart. Third, the latency for the onset of these arrhythmias also may discount the importance of the vasoconstriction or ischemia in this response. In general, the U-46619-induced arrhythmias had an onset time between 8 and 154 s. There was no discernable correlation between the occurrence of the vasoconstriction as deduced from the rise in MABP and the onset of arrhythmias in these experiments.

The lack of measurable reductions in blood flow, absence of ST segment changes, and timing of arrhythmias seem to indicate that the U-46619-induced arrhythmias occur in the absence of significant ischemia in this model and may suggest a role for other mechanisms involved in TXA$_2$-induced arrhythmias. However, it is also possible that any localized, transient changes in coronary blood flow or ischemia were not detected by using microsphere measurements of the ventricle or measurements of ST segment alterations. Likewise, TXA$_2$ induces platelet aggregation, and it is possible that injections of U-46619 may have induced a platelet plug in a small branch of a coronary artery that may have induced these arrhythmias. However, this event would have had to have been small enough to not be detected by total coronary blood flow measurements or alterations of the ST segment. In addition, the arrhythmias were always transient, and no animals ever entered into ventricular fibrillation, which indicates that the platelet plug would have had to have dissolved soon after U-46619 injection. The evidence that some arrhythmias occur very early after injection would also argue against clot formation. Although it is unlikely that platelet aggregation is responsible for the arrhythmias observed in this model, assessment of platelet activity is worthy of future study.

**Influence of autonomic nerves.** Stimulation of sympathetic or parasympathetic nerves secondary to the administration of the TXA$_2$ mimetic could either cause or modulate these observed rhythm disturbances. Specifically, because TXA$_2$ has been shown to stimulate peripheral nerves (13, 20, 21, 48), TXA$_2$ may induce arrhythmias via stimulation of either sympathetic or parasympathetic nerves that innervate the heart.

We first considered the possibility that the sympathetic nervous system played a role in the U-46619-induced arrhythmias. The rationale for this hypothesis was twofold. First, there is a preliminary report that TXA$_2$ stimulates ischemically sensitive sympathetic afferent nerves (13). Second, TXA$_2$ may augment adrenergic neurotransmission specifically by increasing norepinephrine release (41, 42). It has been well documented that excess catecholamine release can induce abnormal heart rhythms and that $\beta$-adrenergic receptor blockade can
reduce these arrhythmias (2, 10, 12, 17, 34). Therefore, we investigated the role of the β-adrenergic receptor in U-46619-induced arrhythmias.

Pretreatment of a series of rabbits with the nonselective β-adrenergic receptor antagonist propranolol (17) did not alter the number of arrhythmias induced by U-46619 compared with the nontreatment group. Absence of a cardiac response to the β-receptor agonist isoproterenol validates the efficacy of the β-receptor blockade. Additionally, we have used doses and protocols similar to previous experiments that have successfully blocked the β-adrenergic receptors (32, 39). Therefore, we conclude that generation of arrhythmias after U-46619 administration occurs in the absence of β-adrenergic receptor stimulation.

We also investigated the role of the parasympathetic nervous system in the development of these arrhythmias. In addition to our findings that TxA2 can stimulate vagal nerves and elicit reflexes, there is additional rationale for analyzing the role of the parasympathetic nervous system in these arrhythmias. The influence of the vagus nerve has been shown to have multiple actions on the heart. Some laboratories have shown that stimulation of the vagus protects the heart from certain arrhythmias (30, 46), whereas other reports indicate that vagal stimulation may contribute to the origin of arrhythmias (22, 38).

We observed a trend that the average number of U-46619-induced arrhythmias was increased in animals that had been pretreated with atropine compared with the nontreatment group. Additionally, U-46619 was injected in a group of animals after bilateral cervical vagotomy. An increase of the number of animals displaying arrhythmias, as well as an increase in the number of arrhythmias in each vagotomized animal compared with the animals with both vagi intact, was observed. Therefore, we conclude that the vagus nerve exerts a protective effect against U-46619-induced arrhythmias.

The increase in the frequency of these arrhythmias after vagotomy or blockade of vagal efferent nerves is not surprising. Weiss et al. (52) demonstrated that increased vagal tone suppressed premature ventricular contractions in a group of human subjects. Prystowsky et al. (35) have shown in humans that tonic vagal activity prolonged the ventricular refractory period and suggested that these changes may reduce arrhythmias (especially ventricular tachycardia produced by a reentry mechanism). Martins and Zipes (26) have provided data showing that vagal stimulation prolongs the effective refractory periods in the ventricle and can antagonize sympathetic activity. Finally there have been several reports demonstrating that acetylcholine can decrease the rate of depolarization in Purkinje fibers (14, 43). On the basis of these findings, it is possible that vagal activity may reduce the U-46619-induced arrhythmias by altering the excitability of the heart tissue.

The observation that the increase in arrhythmias was more pronounced after vagotomy compared with atropine could be due to several events. Although the efficacy of atropine was verified by the absence of responses to acetylcholine at the beginning and at the end of the experiment, it is possible that there was incomplete muscarinic receptor blockade. Another factor is that atropine only eliminates vagal efferent activity, whereas vagotomy obviously interrupts both efferent and afferent vagal traffic. In the presence of atropine, stimulation of vagal afferent nerves may alter central or peripheral neural activity, thereby reducing the responses in the atropine-treated animals compared with responses from the vagotomized animal.

**Direct actions on the heart.** Our findings indicate that significant reductions in coronary blood flow and activation of the autonomic nervous system are not the primary cause of U-46619-induced arrhythmias. Therefore, it is possible that U-46619 may have a direct receptor-mediated effect on cardiac myocytes. Studies conducted using guinea-pig heart tissue revealed that U-46619 induced positive inotropic effects that were independent of α1- or β1-adrenergic receptor blockade and were associated with increased tissue levels of inositol phosphates (25, 37). More recently, Takayama et al. (40) have shown that inflammation-associated tachycardia in mice was mediated by TxA2 and PGF2α (40); the authors found that these effects were mediated by actions of TxA2 and PGF2α on regions of the atrium that contained pacemaker cells.

In studies with isolated cardiomyocytes, U-46619 has been shown to induce changes in calcium dynamics (11, 19) and elicit phosphoinositide turnover and activation of the phospholipase C and IP3 pathway (31). Therefore, it is possible that TxA2 receptors are expressed in cardiac myocytes (23). Recently, our laboratory has identified the presence of TxA2 mRNA in cultured neurons (49), adding support to the possibility that TxA2 may also be expressed in other excitable tissues such as cardiac myocytes. It is possible that stimulation of TxA2 receptors may induce changes in calcium dynamics of myocytes and induce arrhythmias in our in vivo model.

**Significance.** In summary, these results provide further support for the importance of TxA2 in eliciting arrhythmias and may have special significance in cases where the level of TxA2 is elevated above normal. One obvious example is the increase in TxA2 during myocardial ischemia (18, 29). However, another example involves the recent reports that Vioxx, an inhibitor of the inducible form of COX-2, may increase the risk of cardiovascular problems. Under homeostatic conditions a balance likely exists between the levels of TxA2 and prostacyclin (PGI2, a prostaglandin that induces vasodilation and inhibits platelet aggregation). A major source of TxA2 production results from the action of COX-1 located in platelets, while a major source of systemic prostacyclin arises from the enzymatic action of COX-2 located in tissues such as the endothelium (27). Inhibiting COX-2 could potentially reduce the production of PGI2, which would upset the balance between the two eicosanoids in favor of TxA2 and thus augment some of the actions of TxA2 (4, 9, 45). Although it is still unclear as to the exact role of TxA2 in generating the cardiovascular problems associated with Vioxx, our data suggest that the cardiovascular actions of TxA2 may be more complex than simply vasoconstriction and platelet aggregation and deserve further investigation. Although the exact mechanism of TxA2-induced arrhythmias in our model awaits further investigation, it is significant that the presence of arrhythmias after TxA2 receptor stimulation in vivo occurs in the absence of significant reductions in coronary blood flow or activation of the autonomic nervous system.

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