Cyclooxygenase inhibition and baroreflex sensitivity in humans

Kevin D. Monahan and Chester A. Ray

Departments of Medicine (Cardiology) and Cellular and Molecular Physiology, General Clinical Research Center, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

Submitted 13 April 2004; accepted in final form 13 October 2004

Monahan, Kevin D., and Chester A. Ray. Cyclooxygenase inhibition and baroreflex sensitivity in humans. Am J Physiol Heart Circ Physiol 288: H737–H743, 2005. First published October 14, 2004; doi:10.1152/ajpheart.00357.2004.—Animal studies suggest that prostanoids (i.e., such as prostacyclin) may sensitize or impair baroreceptor and/or baroreflex responsiveness depending on the site of administration and/or inhibition. We tested the hypothesis that acute inhibition of cyclooxygenase (COX), the rate-limiting enzyme in prostanoid synthesis, impairs baroreflex regulation of cardiac period (R-R interval) and muscle sympathetic nerve activity (MSNA) in humans and augments pressor reactivity. Baroreflex sensitivity (BRS) was determined at baseline (preinfusion) and 60 min after (postinfusion) intravenous infusion of a COX antagonist (ketorolac; 45 mg) (24 ± 1 yr; n = 12) or saline (25 ± 1 yr; n = 12). BRS was assessed by using the modified Oxford technique (bolus intravenous infusion of nitroprusside followed by phenylephrine). BRS was quantified as the slope of the linear portion of the J) R-R interval-systolic blood pressure relation (cardiovascular BRS) and 2) MSNA-diastolic blood pressure relation (sympathetic BRS) during pharmacological changes in arterial blood pressure. Ketorolac did not alter cardiovascular (19.4 ± 2.1 vs. 18.4 ± 2.4 ms/mmHg preinfusion and postinfusion, respectively) or sympathetic BRS (−2.9 ± 0.7 vs. −2.6 ± 0.4 arbitrary units·beat⁻¹·mmHg⁻¹) but significantly decreased a plasma biomarker of prostanoid generation (plasma thromboxane B₂) by 53 ± 11%. Cardiovascular BRS (21.3 ± 3.8 vs. 21.2 ± 3.0 ms/mmHg), sympathetic BRS (−3.4 ± 0.3 vs. −3.2 ± 0.2 arbitrary units·beat⁻¹·mmHg⁻¹), and thromboxane B₂ (change in −1 ± 12%) were unchanged in the control (saline infusion) group. Pressor responses to steady-state incremental (0.5, 1.0, and 1.5 μg·kg⁻¹·min⁻¹) infusion (5 min/dose) of phenylephrine were not altered by ketorolac (n = 8). Collectively, these data indicate that acute pharmacological antagonism of the COX enzyme does not impair BRS (cardiovascular or sympathetic) or augment pressor reactivity in healthy young adults.

autonomic nervous system; blood pressure; baroreceptor; prostaglandin

THE BAROREFLEX is a powerful negative feedback reflex that responds to and attempts to correct perturbations in arterial blood pressure (BP) (14, 24, 32). The critical role of the baroreflexes in health and disease has received a large amount of attention in the research literature (14). The primary mechanism by which baroreflexes contribute to BP regulation is through influences on both the parasympathetic and sympathetic branches of the autonomic nervous system (14, 24, 32). Specifically, during increases or decreases in BP efferent vagal outflow and/or efferent sympathetic nervous system outflow are altered in an attempt to maintain BP homeostasis. In humans the consequences of impaired baroreflex responses can be profound, and impairment in baroreflex function is recognized in many cardiovascular disease states (14). Accordingly, mechanisms contributing to normal and abnormal baroreflex function are of biomedical significance.

Prostaglandins are prostanoids formed from the conversion of arachidonic acid by the cyclooxygenase (COX) enzyme (34). Animal studies suggest that direct exposure of the carotid sinus to prostanoids such as prostacyclin augments afferent and/or efferent baroreceptor responses to activation and/or deactivation (4, 6, 25, 39). Consistent with these findings, administration of pharmacological substances into the isolated carotid sinus capable of reducing prostanoid synthesis impairs both afferent baroreceptor and efferent baroreflex responses to baroreceptor activation and/or deactivation (4, 6, 25, 39). Collectively, these findings suggest that prostanoids in the carotid sinus sensitize baroreceptor and/or baroreflex responsiveness. In contrast intracardiac administration of prostanoids such as prostacyclin impairs baroreflex responsiveness in animals (28–30, 41) through a vagally dependent mechanism (28, 29, 41). Thus systemic modulation of prostanoid levels may be hypothesized to increase baroreflex sensitivity (BRS) through a facilitory influence on arterial baroreceptors or decrease BRS through impairment in baroreceptor responsiveness mediated by cardiac-related afferents.

Limited data available in humans suggest that baroreflex regulation of heart rate in response to steady-state changes in BP are impaired during systemic administration of a COX antagonist (38). Furthermore, in this same study the pressor responses to intravenous norepinephrine infusion appeared augmented (38). This enhanced pressor reactivity could occur directly by altered sensitivity to vasoactive agents or indirectly through a reduced ability of the baroreflex to buffer such changes. Thus the limited data available in humans suggest that endogenous prostanoids may exert greater biological effects in regions where prostanoids sensitize (i.e., carotid sinus) rather than impair baroreflex and/or baroreceptor responsiveness (i.e., intracoronary). Importantly, in this earlier study only heart rate responses to steady-state BP perturbations were examined, and no direct examination of the effects of endogenous prostanoids on baroreflex regulation of sympathetic nerve outflow was made. This question of how systemic COX antagonism affects baroreflex function is of biomedical interest because of the widespread use of nonsteroidal anti-inflammatory drugs (NSAIDs) (23), which are powerful inhibitors of COX (7, 8). Additionally, reductions in prostanoid levels in animals with cardiovascular disease (hypertension) appear to contribute to impaired baroreflex function (39). Thus it is possible that NSAID ingestion could mimic the effects of cardiovascular disease by impairing baroreflex function secondary to reducing endogenous prostanoid levels.
Accordingly, in the present study we tested the hypothesis that acute pharmacological COX antagonism: 1) impairs baroreflex control of sympathetic outflow and cardiac period and 2) enhances pressor responses to vasoactive medications in humans. To test these hypotheses, BRS was assessed before and after acute intravenous infusion of either saline or a powerful rapidly acting COX antagonist.

**METHODS**

**Subjects**

Twenty-four healthy subjects (17 men and 7 women) were studied in this randomized, double-blinded, placebo-controlled study. All subjects were 18–35 yr of age. Subjects were deemed healthy based on medical history and physical examination, having a resting BP <140/90 mmHg, nonsmoking status, nonobese (body mass index <27 kg/m²), and not taking any medications with known cardiovascular or autonomic nervous system effects. Additionally, plasma creatinine levels were determined to be within the normal range (0.6–1.1 mg/dl) in all subjects before the experimental protocol to avoid concerns of drug toxicity in individuals with impaired renal excretory function.

Written informed consent was obtained from all subjects, and the study was approved by the Pennsylvania State University College of Medicine Institutional Review Board.

**Experimental Design**

Subjects were studied supine and refrained from caffeine ingestion and food consumption for 12 h before testing was started. No subject was regularly taking any NSAID or aspirin and had not ingested either for a minimum of 72 h before the testing.

**Protocol 1: effect of ketorolac on prostanoid synthesis.** The purpose of this protocol was to determine the effectiveness and time course of the reduction in endogenous prostanoid levels after intravenous infusion of ketorolac. In five subjects, 45 mg of ketorolac were intravenously infused. Venous blood samples were withdrawn to document plasma markers of prostanoid synthesis at baseline (i.e., preinfusion) and at 30, 45, 60, 75, and 90 min after infusion.

**Protocol 2: effect of ketorolac on cardiovagal and sympathetic BRS.** The purpose of this protocol was to determine the effect of COX antagonism on BRS. Three BRS trials were performed at baseline (preinfusion). After baseline measurements, subjects (n = 24) were randomly administered a bolus intravenous infusion of either 45 mg of ketorolac in 20 ml of 5% dextrose saline (experimental group; n = 12) or 20 ml of 5% dextrose saline (control group; n = 12) over 30 s. Keterolac is a NSAID that powerfully antagonizes COX (i.e., the rate-limiting step in prostanoid synthesis) (8, 21). Sixty minutes after infusion of either saline or ketorolac, the BRS trials were repeated in triplicate (postinfusion). Fifteen minutes separated each BRS trial in both the pre- and postcondition. BP and heart rate were measured in triplicate before the first BRS trial in both the pre- and postconditions. Additionally, they were assessed ~2 min before each other BRS trial (trials 2, 3, 5, and 6). Resting MSNA was quantified from 3-min recordings obtained during quiet resting conditions immediately before the first trials made in the pre- and postconditions (n = 9; for each the experimental and control group). Both the subject and the investigators were blinded as to which group (i.e., control or experimental) the subject was assigned.

**Protocol 3: effect of ketorolac on pressor responses.** The purpose of this protocol was to determine the pressor responses to steady-state infusions of phenylephrine before and after COX antagonism. In eight subjects BP, cardiac period, and MSNA responses to stepwise steady-state infusions of phenylephrine were determined.

**Measurements**

MSNA. Multifiber recordings of MSNA were obtained by the insertion of a tungsten microelectrode in the peroneal nerve of the leg. A reference electrode was inserted subcutaneously in close proximity to the recording electrode. The recording electrode was adjusted until a site with clear spontaneously occurring sympathetic bursts was established. Standard criteria for acceptable recordings of MSNA were applied (37). Raw nerve recordings were amplified (20,000–70,000 times), filtered (700–2,000 Hz), full wave rectified, and integrated (0.1 s time constant) to obtain mean voltage neurograms.

Cardiovagal and sympathetic BRS. Cardiovagal and sympathetic BRS were assessed using the modified Oxford technique (13, 31). Briefly, nitroprusside was infused intravenously as a bolus (50–100 µg) followed 60 s later by a bolus of phenylephrine (100–150 µg). Data acquisition began ~15 s before nitroprusside infusion and continued for 120 s after phenylephrine infusion. Drugs were first administered at the minimal doses (50 and 100 µg for nitroprusside and phenylephrine, respectively). If the desired effects on systolic blood pressure (SBP) (~15–25 mmHg reduction and subsequent increase from baseline levels) were not obtained, one or both of the drugs doses were increased in 25-µg increments in the subsequent trial until the maximal doses or desired effects on SBP were observed. Fifteen minutes separated all trials even if the drug doses were determined to be insufficient. Initial trials in which the desired effects on SBP were not obtained were not included in the analyses.

Pressor responses to phenylephrine. Pressor and sympathoinhibitory responses were determined during steady-state stepwise infusions of phenylephrine. After a 5-min baseline period, phenylephrine was consecutively infused at rates of 0.5, 1.0, and 1.5 µg·kg⁻¹·min⁻¹ (9, 10). Each dose was infused continuously for 5 min. BP, heart rate, and MSNA were collected continuously.

Arterial BP and heart rate. Resting BP was determined using an automated device (Dinamap XL, Johnson & Johnson). Continuous measurements of BP were made using a Finapres photoplethysmograph (Ohmeda). Heart rate was determined from the ECG.

Blood samples. Venous blood samples were drawn from subjects at baseline and 60 min after either saline or ketorolac infusion for analysis of plasma thromboxane B₂. These measurements were used to document the effectiveness of COX blockade. Samples were collected in prechilled tubes containing EDTA as a preservative and spun in a refrigerated centrifuge. The plasma was then frozen at −80°C until assayed. Thromboxane B₂ levels were quantified by enzyme immunoassay (Amershams Biosciences).

**Data Analysis**

All data were recorded to a Macintosh computer (MacLab 8e, ADInstruments) at 400 Hz for later analyses. An individual unaware of the group or condition associated with the respective data performed all analyses.

MSNA at rest was quantified as bursts per minute and as the sum of the area under individual MSNA bursts expressed as arbitrary units of activity per minute (AU/min). The largest burst at rest was assigned an amplitude of 1,000 AU, and a portion of the nerve recording in which there was neural silence (i.e., no efferent discharges) for at least 5 s was used to set the baseline to zero.

Cardiovagal BRS was quantified as the slope of the linear portion of the R-R interval-SBP relation (binned over 2-mmHg pressure ranges) from the nadir to peak SBP response during each BRS trial (13, 31). Only data contained in the nadir to peak time period were included in the analyses to avoid potential nonlinearities that may exist in cardiovagal baroreflex responses while still allowing characterization of the linear portion of the RR interval-SBP relation (31). A representative regression analysis of the R-R interval-SBP relation is presented in Fig. 1, top. Points clearly falling in either the threshold or saturation region were manually removed from the analysis (18).
sympathetic BRS was quantified as the slope of the linear portion of the MSNA relation (binned over 3-mmHg pressure ranges) from the start of the nitroprusside-induced decrease in diastolic blood pressure (DBP) to the peak DBP response during the trial (13, 31). Because hysteresis is not believed to exist in this relation, the entire period from the start of the nitroprusside-induced decrease in DBP to the peak DBP response was included in the analyses (31). MSNA was quantified as the integrated area underlying a sympathetic burst. Bursts were identified visually after accounting for conduction latency. Any heartbeat not associated with a sympathetic burst was assigned a value of zero and was included in the analysis for the respective DBP bin (13, 31). DBP bins with no sympathetic activity were excluded from the analyses (31). A representative regression analysis is presented in Fig. 1, bottom.

All cardiovascual BRS trials with linear regression coefficients exceeding an r value of >0.70 (31, 35) were averaged together (up to 3), and a single value is reported (preinfusion). Similarly, 60 min after ketorolac or saline infusion all significant cardiovascual BRS trials were averaged and a single value reported (postinfusion). The same process occurred for sympathetic BRS except the criteria for retaining a BRS value was that the r value for the linear regression was >0.50 (2, 31). All significant trials during the pre- and postperiod (up to 3) were averaged together, and a single value was reported for each time point.

Pressor and MSNA responses to steady-state infusion of phenylephrine were determined in the final 2 min of each dose of phenylephrine. Data are expressed as change in the respective measure from those levels determined during the baseline period before the phenylephrine infusion began.

Statistical Analysis

Differences in baseline subject characteristics were determined by t-test, and repeated measures ANOVA was used to determine effects of either ketorolac or saline infusion. Specific contrasts were made using Newman-Keuls post hoc tests. Statistical significance was established as P < 0.05.

RESULTS

Subject Characteristics

Subject characteristics at baseline and after saline (control) or ketorolac infusion (experimental) are shown in Table 1. Subjects in the experimental and control groups were closely matched at baseline.

Effect of Ketorolac on Prostanoid Synthesis

Acute intravenous administration of ketorolac inhibited prostanoid synthesis in all subjects. In a subgroup of subjects (n = 5), we noted that thromboxane B2, a marker of prostanoid synthesis, was equally reduced from baseline resting levels at 30 (−52 ± 12%), 45 (−56 ± 13%), 60 (−56 ± 10%), 75 (−57 ± 10%), and 90 min (−57 ± 11%) after infusion of ketorolac. These data establish that 1) ketorolac is an effective COX antagonist and 2) that COX antagonism was present throughout the period in which the postketorolac infusion measures were made in this study. BRS trials (postinfusion) were made at time points approximately equal to the 60-, 75-, and 90-min values.

Effect of Ketorolac on Cardiovascular and Sympathetic BRS

At baseline, cardiovascual and sympathetic BRS were similar in both groups (Fig. 2). Because it was difficult to obtain an adequate MSNA recording site, sympathetic BRS was only measured in 9 of 12 subjects in each the experimental and control group. Cardiovascual BRS was measured in all 12 subjects in each group. BP and heart rate were similar before each BRS trial in both the experimental and control group (Table 2). Administration of ketorolac in the experimental group did not alter cardiovascual BRS (19.4 ± 2.1 vs. 18.4 ± 2.4 ms/mmHg for baseline and after ketorolac infusion, respectively; Fig. 2)

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>Post</th>
<th>Control</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>9/3</td>
<td>8/4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>24±1</td>
<td>25±1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>175±2</td>
<td>176±3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>73.1±3.2</td>
<td>75.1±4.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.7±0.7</td>
<td>24±1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>112±2</td>
<td>112±3</td>
<td>118±4</td>
<td>118±3</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>65±2</td>
<td>66±3</td>
<td>65±2</td>
<td>65±2</td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>60±2</td>
<td>60±2</td>
<td>59±2</td>
<td>58±2</td>
<td></td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>15±2</td>
<td>16±3</td>
<td>16±4</td>
<td>15±2</td>
<td></td>
</tr>
<tr>
<td>Total MSNA, AU/min</td>
<td>1,380±189</td>
<td>1,646±302</td>
<td>1,737±364</td>
<td>1,874±331</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12 subjects for the preexperimental and precontrol values. BMI, body mass index; BP, blood pressure; MSNA, muscle sympathetic nerve activity; AU, arbitrary units.
The primary new finding from the present study is that acute systemic infusion of a COX antagonist, which suppresses or sympathetic BRS ($-2.9 \pm 0.7$ vs. $-2.6 \pm 0.4$ AU·beat$^{-1}$·mmHg$^{-1}$; Fig. 2) but reduced thromboxane B$_2$ levels (change in $-53 \pm 11\%$; $P < 0.05$ from levels measured at the beginning of the protocol). Similarly cardiovagal ($21.3 \pm 3.8$ vs. $21.2 \pm 3.0$ ms/mmHg; Fig. 3) and sympathetic BRS ($-3.4 \pm 0.2$ vs. $-3.2 \pm 0.2$ AU·beat$^{-1}$·mmHg$^{-1}$; Fig. 2) were unchanged in the control group. Thromboxane B$_2$ levels were unchanged in the control group (change in $-1 \pm 12\%$) 60 min after ketorolac infusion. Related to the above findings, the following data are important to consider. First, the total number of BRS trials performed during the entire protocol was similar in the experimental ($6.7 \pm 0.3$) and control groups ($6.3 \pm 0.3$). Second, the average number of slopes used in the generation of the mean values for each expression of BRS did not differ between groups and was between $2.0 \pm 0.2$ and $2.6 \pm 0.2$ trials at baseline and after infusion of ketorolac or saline. Third, the mean correlation coefficient for the BRS trials that met the retainment criteria and therefore were used to generate the reported cardiovagal and sympathetic BRS slopes was high in both the experimental ($r = 0.84 \pm 0.05–0.94 \pm 0.04$) and control groups ($r = 0.81 \pm 0.05–0.93 \pm 0.04$). Fourth, the likelihood that a trial would meet the retainment criteria did not depend on its order (i.e., the last trial for baseline or after ketorolac or saline infusion was as likely as the first trial during that condition to meet the retainment criteria). Finally, there was no evidence of any time-specific changes in BRS or any effect of repeated trials on BRS values when data were examined on a trial-by-trial basis (all $P > 0.50$).

In a subgroup of subjects ($n = 10$) we reanalyzed the baseline sympathetic BRS trials to include only 1) the nitroprusside-induced decrease in BP, 2) the increasing period of BP, and 3) the entire period from the beginning of the nitroprusside-induced decrease in BP to the peak response after phenylephrine (i.e., increasing and decreasing BP values) to exclude the possibility that nonlinearities or hysteresis may exist in sympathetic BRS. The results of these reanalyses indicated that sympathetic BRS was the same for decreasing ($-2.8 \pm 4.7; r = 0.67$), increasing ($-3.1 \pm 3.0; r = 0.78$), and decreasing and increasing pressures combined ($-2.9 \pm 1.6; r = 0.82$).

**Effect of Ktorolac on Pressor Responses**

Incremental infusion of phenylephrine elicited a dose-dependent increase in mean BP and cardiac period ($n = 8$) and inhibition in MSNA ($n = 6$) before (preinfusion) and after (postinfusion) intravenous infusion of ketorolac (Fig. 3). Changes in these physiological variables did not differ from preinfusion values when measured 60 min after ketorolac infusion (Fig. 3).

**Correlates**

At baseline, neither cardiovagal nor sympathetic BRS was significantly related to thromboxane B$_2$ levels. Additionally, no correlation was noted between the magnitude of change in thromboxane B$_2$ and either sympathetic or cardiovagal BRS in the subjects.

**DISCUSSION**

The primary new finding from the present study is that acute systemic infusion of a COX antagonist, which suppresses...
endogenous prostanoid synthesis, does not impair cardiovagal or sympathetic BRS in healthy young adults. This apparent lack of effect of endogenous prostanoids on baroreflex function contrasts prior data collected in animals examining region-specific (e.g., carotid sinus and cardiac) effects of prostanoids on baroreceptor and/or baroreflex function. Moreover, the present study demonstrates that acute systemic COX antagonism does not enhance pressor responses to phenylephrine.

Phenylephrine is the biological precursor for prostanoid synthesis (e.g., prostaglandins and thromboxanes) generation (34). This conversion of arachidonic acid is dependent and rate limited by cyclooxygenase (COX) (8, 21). Reductions in prostanoid synthesis can be measured in a variety of manners but usually involves measuring plasma or regional levels of stable breakdown products of prostaglandins (i.e., 6-keto-PGF\textsubscript{1α} or immunoreactive prostaglandin E\textsubscript{2}) or thromboxanes (i.e., thromboxane B\textsubscript{2}) (16, 26, 40). Because both prostaglandin and thromboxane synthesis is COX dependent, either measurement can be used as a bioassay of COX antagonism (i.e., prostanoid synthesis inhibition). Consistent with these statements, we demonstrate a pronounced decrease (change in −53%) in plasma thromboxane B\textsubscript{2} levels 60 min after intravenous infusion of a large dose (45 mg) of ketorolac. These reductions are greater than our previous demonstration using an oral dose of ketoprofen in young adults (change in −40%) (11). These data confirm that intravenous infusion of ketorolac provides an appropriate model to examine acute reduction in prostanoid synthesis in vivo in humans.

Data from studies using an isolated carotid sinus preparation suggest that prostanoids such as prostacyclin sensitize baroreceptor and baroreflex responsiveness (3–6, 25, 39). These data indicate that prostanoids may play a pathological role in impaired baroreceptor and/or baroreflex function in conditions associated with altered prostanoid synthesis (39). Additionally, endothelial denudation impairs baroreceptor sensitivity (4). This finding suggests that a product of the vascular endothelium, such as locally released prostanoids, contributes to normal baroreceptor function. Moreover, it suggests that endothelial dysfunction may contribute to altered baroreflex function. Consistent with this concept, hypertension is associated with impaired baroreflex sensitivity (17) and endothelial dysfunction (27), which may be related (39).

In contrast to the sensitizing effect intracarotid prostanoids exert on baroreceptor and/or baroreflex responsiveness, intracoronary infusion of prostanoids appears to inhibit baroreflex responses. Specifically, left circumflex coronary artery infusion of prostacyclin impairs baroreflex control of renal sympathetic nerve activity and heart rate (30, 41). Additionally, acute coronary artery occlusion, which increases prostaglandin synthesis (1), also impairs baroreflex control of renal sympathetic nerve activity (42). Importantly, this inhibition is prevented by prior administration of a COX antagonist (42). Consistent with this finding, other studies have demonstrated that intracoronary infusion of prostaglandin E\textsubscript{2} and arachidonic acid impair baroreflex control of heart rate (29). It is important to emphasize that all of these previous studies examining the effects of intracardiac administration of prostaglandins on baroreflex function have either exogenously administered prostanoids or utilized COX antagonism only after elevating prostanoid levels well above those levels observed at rest. The influence that basal generation of prostanoids exerts on baroreflex function in healthy well-perfused cardiac tissue has not been determined. Myocardial production of prostaglandins is low in healthy myocardium (33), which suggests that elevated prostanoid levels may be prerequisite for observing an effect of intracardiac prostanoids on baroreflex function.

Intravenous administration of prostanoids is likely to provide a more integrative response to prostanoids, as systemic circulation should occur. However, studies examining the effects of systemic administration of prostanoids have provided

![Fig. 3. Changes in mean arterial blood pressure (MAP), cardiac period (R-R interval), and MSNA (both bursts/min and change in %total MSNA) during steady-state infusion of phenylephrine before (Pre; filled circles) and after (Post; open circles) infusion of ketorolac. Phenylephrine caused a dose-dependent increase (Post; open circles) infusion of ketorolac. Phenylephrine was administered consecutively at doses of 0.0 (baseline), 0.5, 1.0, and 1.5 µg·kg\textsuperscript{-1}·min\textsuperscript{-1} for 5 min each. Data were obtained during the final 2 min of each dose of phenylephrine.](http://ajpheart.physiology.org/288/2/Fig3.jpg)
inconsistent results. Intravenous infusion of prostacyclin impairs baroreflex regulation of heart rate in conscious dogs (28). These data suggest that those effects exerted by prostanooids in regions that impair baroreflex responsiveness, such as in the heart, may be more biologically important than those effects exerted in regions that sensitize baroreceptor and/or baroreflex responses, such as in the carotid sinus. However, the effects of exogenous intravenous administration of prostanooids on baroreflex function have not consistently been shown to impair baroreflex control of heart rate (29) or renal sympathetic nerve activity (19). These inconsistent results may be explained by the fact that prostanooids are removed from the blood by many organs or tissues as they are circulated (12, 15). This could raise concerns over whether the exogenously infused prostanooids reached the target tissues in which they could influence autonomic control (i.e., the heart or carotid sinus). Antagonizing prostanooids systemically should overcome this limitation because the COX antagonists will circulate systemically and exert their influence on all tissues.

The discussions so far suggest that prostanooids may modulate baroreceptor and/or baroreflex function. Moreover, they indicate that the direction of this modulation may be critically dependent on the site of administration. Only one previous study has examined the effects of COX antagonism on baroreflex function in humans. In this study, oral administration of the NSAID indomethacin blunted the decrease in heart rate in response to increases in BP (38). However, the results of this study must be interpreted cautiously for several reasons. First, stepwise steady-state infusions of norepinephrine were used to assess reflexive changes in heart rate. These stepwise infusions of vasoactive medication may produce baroreceptor resetting or adaptation (14) as well as chronotropic effects that include both parasympathetic and sympathetic components (22). This may explain why methods utilizing steady-state drug infusions compared with bolus infusions of vasoactive medications, which result in primarily vagally mediated changes in cardiac period, do not correlate (36). Second, the vasoactive medication used to increase BP was norepinephrine. Use of norepinephrine confounds the results of the study as it exerts direct effects on the primary outcome measure (i.e., heart rate) as well as possible direct and indirect influences on baroreceptors (3). Third, no measure of reflex control of sympathetic outflow was made leaving unanswered the effects of endogenous prostanooids on its control. Collectively, based on these limitations it cannot be determined what role endogenous prostanooids exert on the baroreflex control in humans.

In the present study it was not possible to selectively alter prostaglandin levels in regions in which prostaglandins may exert specific effects (e.g., selectively in the coronary circulation or in the carotid sinus). Importantly, region-specific alterations in autonomic control via prostanooids have never been examined in humans. Whether region-specific modulation of prostaglandins modulates any measure of autonomic and/or baroreflex control directly in humans appears to warrant future study. The examination of these effects may explain why our results differ from prior data obtained in experimental animals (3, 4). In this context it is unclear how prostanooid levels in areas critical to baroreflex functioned such as in the carotid sinus compared between prior animal studies and our study. It is possible that these levels differed and contributed to our findings. Moreover, we were unable to selectively modulate specific prostanooids in the present study, because ketorolac is a nonselective COX antagonist. However, importantly the present study does provide insight into the systemic integrative response to acute pharmacological COX antagonism in humans.

This integrative examination of baroreflex function after altering prostanooid synthesis may be particularly important due to the large number of NSAIDs available through both prescription and over-the-counter mechanisms. Presently, we are not aware how prevalent use of such substances is, but prior estimates exceeded 100 million prescriptions annually (23). This number is likely to be much larger today with the development of new NSAIDs as well as if over-the-counter use is considered. The present findings do not support a role of acute intake of NSAIDs on alterations in baroreflex regulation of either cardiac period or MSNA. However, we cannot exclude a role of alterations in prostanooid synthesis in altered baroreflex function associated with more chronic ingestion of NSAIDs or in disease states associated with chronic alterations in prostanooid synthesis. Thus it may be insightful to administer a COX antagonist in patient populations with pathologies such as ischemic heart disease in which prostanooid levels may be affected (20) to determine their role in baroreflex responsiveness.

The present data do not support the hypothesis that pressor responses to phenylephrine are augmented after acute COX antagonism. Earlier findings demonstrated enhanced pressor response to intravenous infusion of norepinephrine after COX antagonism (38). The reason for these discrepant findings are unknown but may result from the fact that norepinephrine exerts more complex cardiac and vascular effects. The former being mediated by both by chronotropic and ionotropic effects and the latter by the balance between α-adrenergic receptor-mediated vasoconstriction and β-adrenergic receptor-mediated vasodilation. Therefore, it is difficult to identify which factor(s) mediated the enhanced pressor reactivity in this earlier study (38). We believe these effects are related to the drugs used to elicit the pressor response (phenylephrine versus norepinephrine).

Several limitations are associated with the present study. First, ketorolac-induced decreases in endogenous prostanooids, measured as a reduction in plasma thromboxane B2, were incomplete. Therefore, we cannot determine whether complete suppression of endogenous prostanooids would impair BRS in healthy young humans. It is important to reemphasize that thromboxane B2 levels were reduced by >50%, suggesting that significant COX antagonism and ensuing reductions in prostanooids does not impair BRS in healthy young adults. Second, with the present experimental design, we cannot determine whether increasing prostanooids would influence BRS or pressor reactivity.

In conclusion, these data suggest that an acute systemic COX antagonism does not impair cardiocagal or sympathetic BRS in healthy young humans. Moreover, pressor responses to the vasoactive drug phenylephrine are not enhanced after ketorolac infusion. These findings suggest that acute intake of NSAIDs (COX antagonists), such as ketorolac, are unlikely to alter autonomic regulation of BP or pressor responsiveness to drugs, such as phenylephrine in humans.
REFERENCES


