The Influence of Indomethacin on Blood Pressure During the Infusion of Vasopressors

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SUMMARY The effect of indomethacin and its vehicle on blood pressure was studied in conscious rabbits during the infusion of three vasopressors. The cyclooxygenase inhibitor raised mean arterial pressure 12 (vehicle: 3) mm Hg during norepinephrine infusion, 5 (vehicle: 0) mm Hg during angiotensin II infusion, and 5 (vehicle: -8) mm Hg during arginine vasopressin infusion. When saline was given in place of vasopressors, indomethacin failed to alter blood pressure. Since indomethacin elevated pressure in the presence, but not the absence, of all three vasopressors, the possibility that elevation of blood pressure per se stimulates synthesis of vasodilator prostaglandins was considered. A pressor action of indomethacin was observed in ganglion-blocked animals, in which absolute blood pressure remained below normotensive levels during angiotensin II infusion. Thus, indomethacin raised arterial pressure during the infusion of norepinephrine, angiotensin II, and vasopressin, and this action was not influenced by manipulation of blood pressure. These results suggest that each vasopressor promotes prostaglandin synthesis independently to a degree sufficient to restrain its pressor action. (Hypertension 8: 772-778, 1986)

KEY WORDS • prostaglandins • rabbit

ANGIOTENSIN II (ANG II), norepinephrine (NE) and arginine vasopressin (AVP) play a central role in the regulation of arterial blood pressure, and all three affect the production of vasodilator prostaglandins (PGs). For example, they stimulate the formation of arachidonic acid metabolites in the kidney, and inhibition of PG synthesis potentiates their renal vasoconstrictor actions. Similar relationships have been demonstrated in other tissues; inhibition of PG synthesis enhances vasoconstrictor responses to NE in splenic, cutaneous, and mesenteric vasculatures and to ANG II in the heart and uterus.

Available evidence, then, argues favorably for a compensatory role of PGs in the control of local blood flow when levels of vasoconstrictor hormones are increased; however, the impact of these local relationships on the regulation of arterial blood pressure is unclear. Currently, the relationship of vasopressors, PGs, and the regulation of arterial blood pressure is not well defined. Several reports suggest that PGs attenuate increases in blood pressure induced by NE or ANG II in humans or animals with elevated plasma renin activity. Evidence for a similar relationship in normal physiological states is fragmented. Some reports indicate that cyclooxygenase inhibitors enhance the pressor response to ANG II in normal animals, while others do not. The negative data are concealed in the control groups of studies focusing on animals with a compromised renin-angiotensin system. For example, cyclooxygenase inhibitors amplify the ANG II pressor response in pregnant rabbits and sodium-restricted rats and the response to NE in pregnant rabbits, but all studies report no effect in the physiologically intact control groups. The concept that PGs modify vasopressor responses is not new, nor is it surprising in hyperreninemic conditions, in which increased PG synthesis could be the consequence, or the instrument, of renin secretion. These vasopressor-PG relationships are unique, and attempts to draw general conclusions are presumptive. The nature of such a relationship in normal animals is contentious and merits closer scrutiny.

The present study comprehensively examined how a cyclooxygenase inhibitor influences the blood pressure...
of normal conscious rabbits in the presence of three different classes of vasopressor: NE, ANG II, and AVP. Indomethacin treatment was superimposed during a constant infusion of vasopressor, and the strength of the experimental design was the use of powerful repeated-measures analyses, which permit the detection of anticipated small differences. The hypothesis that changes in blood pressure per se stimulate PG synthesis, independently of prevailing levels of vasopressors was tested by comparing the effect of indomethacin on the vasopressor response in normal animals with that in animals in which absolute blood pressure levels were manipulated with a ganglion blocker.

Materials and Methods
Seventy-four male, New Zealand White rabbits (2.3–3.4 kg) were maintained on Wayne rabbit ration (Chicago, IL, USA) and water ad libitum. The chow contained 130 mEq sodium and 360 mEq potassium, and sodium intake was estimated at 20 mEq/day. Experiments were performed on conscious animals placed in a restraining box, and surgical preparation was conducted using a local anesthetic, lidocaine (Xylocaine; Astra Pharmaceutical Products, Worcester, MA, USA). Procedures were conducted in accordance with institutional guidelines governing animal use. A polyethylene cannula (PE-50; Clay-Adams, Parsippany, NJ, USA) was filled with heparinized saline (40 U/ml), inserted into a central ear artery, and connected to a Statham pressure transducer (Model P23Db; Oxford, CA, USA). Arterial blood pressure and heart rate were recorded using a Grass polygraph (Model 7D; Quincy, MA, USA). Drugs were infused by means of 23-gauge needles placed into each marginal ear vein; the vasopressor was given in one ear (0.1 ml/min), and all other drugs were infused into the other ear. Preparations of [Hle\(^3\)]ANG II (1106 g/mol, Sigma, St. Louis, MO, USA) and AVP (Sigma) were diluted in 0.9% sodium chloride immediately before use, and NE bitartrate (346 g/mol; Sigma) was diluted with a solution containing ascorbic acid (88 mg/L) and 0.9% sodium chloride. Indomethacin (Sigma), or its vehicle (sodium carbonate), was also prepared immediately before use and administered in a volume of 4 ml statim, followed by a 30-minute infusion at a rate of 0.1 ml/min.

The efficacy of indomethacin (10 mg/kg statim, followed by a 30-minute infusion at a rate of 50 \(\mu g/kg/min\)) as a cyclooxygenase inhibitor was confirmed by the observation that it abolished the depressor response to a 5-minute infusion of arachidonic acid (400 ng/kg/min i.v.; Sigma). The dose is high relative to those reported in most studies, but reduction by half failed to abolish the arachidonic acid response. The rabbit, unlike other species, tolerates the dose well; the efficacy of the drug might vary among species and appears to differ markedly according to target tissue. \(^{11}\)

The NE (6 \(\mu g/kg/min\)), ANG II (50 ng/kg/min), and AVP (100 mU/kg/min) were infused for 60 minutes in three separate groups of animals. After 30 minutes, a priming dose of indomethacin was given (10 mg/kg), followed by an infusion (50 \(\mu g/kg/min\)) that was also terminated at 60 minutes. Three additional groups of rabbits received the identical vasopressor infusions, but vehicle administration was substituted for indomethacin. One more group received indomethacin, but isotonic saline was given in place of vasopressor. Recordings of mean arterial blood pressure and heart rate were obtained in all seven groups of animals during a preinfusion control phase, vasopressor alone, vasopressor with indomethacin or vehicle, and a postinfusion phase.

Additional studies were performed to determine if the effects of indomethacin relate directly to prevailing levels of vasopressor or, perhaps, to the induced elevation of blood pressure per se. In these experiments, ANG II was infused into rabbits treated with a ganglion blocking drug. The manipulation resulted in a pressor action of the peptide, in which absolute blood pressure remained below the original baseline level of the untreated animals. The dose of ANG II was reduced to 10 ng/kg/min to satisfy this criterion. Three experiments were performed as described previously, with the following exceptions. Intravenous administration of 1 mg/kg of chlorisondamine chloride (CIBA Pharmaceutical, Summit, NJ, USA) preceded ANG II infusion by 20 minutes. Cardiovascular data were obtained before and after ganglion blockade. This was accomplished in one group given indomethacin and in one not given indomethacin. A third group was given indomethacin but not chlorisondamine.

Results are expressed as mean ± SEM. Mean arterial blood pressure recordings obtained at designated time points during the infusion of the vasopressors were processed by a one-way analysis of variance test for repeated measures and a modified Newman-Keuls test. \(^{12}\) Since all experiments are designed to include indomethacin-treated plus separate vehicle-treated groups, further analyses were performed to determine interaction. This was accomplished by using a two-way analysis of variance test with repeated measures on one element. \(^{12}\) The second element, without repeated measures, represents the level of treatment between groups. Heart rate data obtained from each phase of the experiments were processed by one-way analysis of variance as just described.

Results
Figures 1, 2, and 3 illustrate the mean arterial blood pressure responses to NE (6 \(\mu g/kg/min\)), ANG II (50 ng/kg/min), and AVP (100 mU/kg/min), respectively. Each figure describes two experiments: one including indomethacin treatment and a control experiment in which vehicle was substituted for indomethacin.

To facilitate quantitative description in the text, a single value for the change in pressure after treatment (with indomethacin or vehicle) is derived from the difference between the means of the final blood pressure value recorded in each infusion phase, but the statements of significance relate to multiple compari-
FIGURE 1. Effect of indomethacin, 10 mg/kg statim followed by 50 μg/kg/min for 30 minutes (n = 7), and vehicle (n = 7) on mean arterial pressure during norepinephrine (NE) infusion. Values are expressed as mean ± SEM, and zero minute corresponds to initiation of the NE infusion. Recordings obtained during NE infusion are highlighted, and asterisks denote individual mean differences determined by one-way analysis of variance and a modified Newman-Keuls test (p<0.01). Two-way analysis of variance indicated a significant interaction between the two experiments (p<0.05). Differences in heart rate (HR) are indicated; each letter denotes a significant difference from all means in the period not designated by the same letter (p<0.05).

FIGURE 2. Effect of indomethacin, 10 mg/kg statim followed by 50 μg/kg/min for 30 minutes (n = 6), and vehicle (n = 8) on mean arterial pressure during angiotensin II (ANG II) infusion. Values are expressed as mean ± SEM, and zero minute corresponds to initiation of the ANG II infusion. Recordings obtained during ANG II infusion are highlighted, and the asterisk denotes individual mean differences determined by one-way analysis of variance and a modified Newman-Keuls test (p<0.05). Two-way analysis of variance indicated a significant interaction between the two experiments (p<0.05). Differences in heart rate (HR) are indicated; each letter denotes a significant difference from all means in the period not designated by the same letter (p<0.05). ANG II = ANG II.

The results obtained during AVP infusion convey a different pattern. Despite constant infusion of a large dose of the peptide, it was not possible to maintain a steady blood pressure elevation. This was observed also in pilot experiments with various doses of AVP and is reflected by a progressive blood pressure decay in the vehicle infusion experiment (see Figure 3). Thus, significant changes were detected in both indomethacin-treated and vehicle-treated control groups. Blood pressure fell in the control group (-8 mm Hg) compared with a rise (5 mm Hg) in the indomethacin-treated group. The interaction was significant (p<0.0001), and in real terms, the pressor effect of indomethacin during AVP infusion might be given more appropriately as the difference between these two values (13 mm Hg).

The results illustrated in Figure 4 indicate that pressor effects of indomethacin are not manifest in the...
absence of vasopressor in this model. When saline infusion was given in place of vasopressor, the change in blood pressure after indomethacin (1 mm Hg) was not significant.

Corresponding heart rate data are given in Figures 1 through 4. A slight decline with time was evident in the absence of vasopressor, and indomethacin showed no consistent effect on heart rate. Marked bradycardia was observed during the NE and AVP infusions, but not during the ANG II infusion.

Data were assimilated from all three experiments with vasopressors and indomethacin. Individual blood pressure changes resulting from indomethacin treatment were corrected for the mean changes in control experiments and compared as is, or as the logarithmic function, with the corresponding cardiovascular data obtained in the preceding vasopressor infusion phase. Regression analysis of the data illustrated in Figure 5 revealed a direct relationship between the indomethacin response and the vasopressor-induced bradycardia ($r = +0.57, p < 0.02$; log transform: $r = +0.60, p < 0.02$). Although it is tempting to rationalize that the pressor and bradycardic response to vasopressor vary pari passu and thus both should exhibit a positive relationship with the indomethacin effect, the indomethacin effect correlated inversely ($r = -0.51, p < 0.025$; log transform: $r = -0.61, p < 0.005$) with the vasopressor-induced increase in blood pressure (see Figure 5).

The results illustrated in Figure 6 show the effect of indomethacin and chlorisondamine on the ANG II (10 mg/kg/min) pressor response. The top panel confirms that indomethacin exerts a pressor action (3 mm Hg, $p < 0.01$) in the presence of this reduced dose of the peptide. Rabbits given chlorisondamine showed a rise in pressure of 9 mm Hg after indomethacin (middle panel) and of 2 mm Hg after administration of the vehicle for indomethacin (bottom panel). A posteriori analysis of the ganglion-blocked animals revealed that all preindomethacin and postindomethacin blood pressure values were significantly different ($p < 0.01$), while the vehicle-treated group showed isolated differences among means. The tendency for pressure to rise in the latter group is presumably a result of the volume load of the vehicle manifesting an action in the absence of normal cardiovascular reflexes. Making allowances for this effect, the rise in pressure after indomethacin in the first two experiments is comparable. Thus, indomethacin produced a similar pressor action with or without ganglion blockade despite a difference of 20 mm Hg in absolute blood pressure, findings that fail to support the hypothesis that cardiovascular status per se directly influences arachidonic acid metabolism.
Chlorisondamine administration was associated with tachycardia, and heart rate continued to rise during ANG II infusion despite an increase in pressure (see Figure 6). Indomethacin had no discernible effect on heart rate.

Discussion

The results of the present study demonstrate that indomethacin exerts a pressor action in the conscious rabbit during the infusion of NE, ANG II, or AVP. In the absence of vasopressors, however, the cyclooxygenase inhibitor does not influence blood pressure. One might infer from these data that baseline blood pressure is not affected by PGs but that infusion of vasopressors results in the stimulation of PG synthesis to a degree sufficient to modify vasopressor action. The efficacy of this dose of indomethacin as an inhibitor of vasodepressor PG synthesis was confirmed. Actions of indomethacin unrelated to cyclooxygenase inhibition are unlikely since previous studies in this laboratory demonstrated that sodium meclofenamate, a structurally dissimilar inhibitor, modifies the cardiovascular response to ANG II in an identical fashion.13 Reports showing that cyclooxygenase inhibition elevates blood pressure in normotensive animals14 and humans15 contrast with reports that they do not.16-18 Similar conflicts are evident in various forms of human and experimental models of hypertensive disease.18 Furthermore, some studies demonstrate that cyclooxygenase inhibitors actually reduce arterial blood pressure when plasma renin activity is elevated.19,20 The equivocal data have prompted attempts to dissect the interaction between PGs and pressor mechanisms, but the majority of experiments have been conducted in humans or animals with elevated plasma renin activity. Cyclooxygenase inhibitors potentiate the pressor re-

FIGURE 5. Relationship between the pressor response to indomethacin and the initial cardiovascular response to vasopressors. Data points represent angiotensin II (△), norepinephrine (●), and arginine vasopressin (○).

FIGURE 6. Effect of indomethacin (10 mg/kg statim followed by 50 μg/kg/min for 30 minutes) on arterial pressure during the infusion of angiotensin II (ANG II) in ganglion-blocked rabbits. Top panel: indomethacin without chlorisondamine (n = 8). Middle panel: indomethacin plus chlorisondamine (n = 8). Bottom panel: chlorisondamine without indomethacin (n = 8). Values are expressed as mean ± SEM, and zero minute corresponds to the initiation of the ANG II infusion. Recordings obtained during ANG II infusion are highlighted, and asterisks denote individual mean differences determined by one-way analysis of variance and a modified Newman-Keuls test (p < 0.01). The following interactions were determined by two-way analysis of variance: indomethacin plus chlorisondamine versus indomethacin minus chlorisondamine (p < 0.001); indomethacin plus chlorisondamine versus chlorisondamine minus indomethacin (p < 0.0001); chlorisondamine minus indomethacin versus indomethacin minus chlorisondamine (not significant). Heart rate is given as mean ± SEM; each letter denotes a significant difference from all means in the period not designated by the same letter (p < 0.05). ALL = ANG II.
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spontaneous response to ANG II in sodium-restricted and pregnant humans.21,22 In patients with chronic autonomic failure or cirrhosis and ascites,23 24 and in anesthetized dogs,25 Similarly, pressor responsiveness to NE was increased by indomethacin in patients with Bartter's syndrome26 or chronic autonomic failure.23 Under basal physiological conditions, data are equivocal. Cyclooxygenase inhibitors augment the pressor action of NE in the conscious rat27 and humans,28 and of ANG II in normal humans29 and conscious rabbits.30 31 Other studies comparing normal animals and animals with presumed elevated plasma renin activity conflict. Cyclooxygenase inhibitors failed to alter the ANG II or NE pressor response in control groups under basal physiological conditions.8-10 The present study offers a comprehensive examination of three vasopressors, in which the experimental design and a powerful statistical approach permit the detection of very small changes in blood pressure. The data confirm modest effects of indomethacin during infusion of vasopressors, and it might be reasonable to speculate that the effect is heightened during the infusion of NE or ANG II in altered physiological states.

Indomethacin elevated blood pressure during the infusion of all vasopressors studied without affecting heart rate, but the responses revealed some unanticipated associations with the initial cardiovascular response to vasopressor. The magnitude of the initial pressor response followed the order: ANG II > NE >> AVP, under the defined conditions and doses described, yet the bradycardic response followed the order: AVP > NE >> ANG II. These observations are not consistent with simple cardiovascular reflexes, nor are they explicable in terms of the expected inherent chronotropic actions of the three vasopressors. The blood pressure response to indomethacin revealed a negative correlation with the magnitude of the original response to vasopressor and a positive correlation with the bradycardic response. Interpretation of the observations is elusive, but consider a provocative postulate centered on the properties of PGI, a potent vasodilator synthesized by arterial blood vessels; PGI, can promote bradycardic,27 and plasma levels correlate tightly with depressor aspects of the ANG II response.13 Thus, for relatively high rates of PGI, synthesis, we might predict an increased pressor response to indomethacin and an initial cardiovascular response to vasopressor that is characterized by a relatively reduced pressor action and enhanced bradycardia. The rationale is speculative and is not presented to exclude consideration of other major factors, but it does offer a cohesive and attractive explanation of the unusual observations.

Considerations outlined above might offer a clue to the progressive decay of the pressor response to AVP, an event reversed by indomethacin. The AVP-induced deterioration of cardiac performance has been reported elsewhere,28 and simple cardiovascular reflexes cannot account for the pronounced bradycardia observed in this study. Marked and sustained PGI production in response to AVP, and subsequent reduction of heart rate and vascular resistance in concert, could be a significant factor in the gradual normalization of blood pressure.

The qualitatively similar effects of indomethacin in the presence of all three vasopressors pose the following question: Is stimulated PG synthesis, and consequent antagonism of the pressor action, a common feature of vasopressor excess? Further, if such a feedback loop is implicated in the regulation of arterial blood pressure, what is the nature of the stimulus for PG synthesis? Direct receptor-mediated actions specific to each vasopressor represent one hypothesis, but the notion that changes in blood pressure per se affect arachidonate metabolism also should be considered. Piper and Vane29 suggested that cellular distortion was a common feature of various types of stimuli for PG release from the lung. Since vascular endothelium synthesizes PGS,30 one might conjecture that mechanical events associated with changes in arterial blood pressure (i.e., the denoting feature of vasopressor action) directly influence local PG production by arterial resistance vessels. The final experiments described in this study were designed to address this hypothesis. The presence of an indomethacin effect in intact animals, but not in the hypotensive ganglion-blocked model, would support the hypothesis that the level of blood pressure per se is a determinant of PG production. In contrast, the experiments revealed that indomethacin raised blood pressure in both groups; results consistent with a specific effect of ANG II on PG production and, by inference, consistent with specific stimulation by NE and AVP.

In conclusion, the capacity of indomethacin to elevate blood pressure in the presence, but not the absence, of NE, ANG II, and AVP suggests that the pressor actions of these compounds are associated with increased synthesis of vasodepressor PGS, which curtail the pressor action. The effect appears to be attributable to specific actions of each vasopressor, rather than their common action, to increase blood pressure per se. The physiological significance remains vague, but the data suggest that the integrity of arachidonic acid metabolism represents a component of blood pressure regulation, and a defect might exacerbate hypertensive disease associated with overproduction of NE, ANG II, or AVP. In this regard, it is interesting to note that rabbits with induced perinephritic hypertension exhibit a defect in depressor mechanisms31 that appears to be related to PG metabolism.13

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