PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **180**, 258–263 (1985)

Prostaglandin Modulation of the Vascular Effects of Vasopressin in the Conscious Rat (42173)

BENJIMEN R. WALKER

Department of Physiology, Tulane University School of Medicine, New Orleans, Louisiana 70112

Abstract. Experiments were performed on conscious, male Sprague–Dawley rats to determine whether cyclooxygenase inhibition affects the pressor response to exogenous vasopressin. The rise in arterial blood pressure was tested in response to 1.0, 2.5, 5.0, and 12.5 mU synthetic arginine vasopressin both before and following cyclooxygenase inhibition with either meclofenamate or the structurally dissimilar inhibitor ibuprofen. In addition, time control experiments were also performed where only the saline vehicle for the drugs was administered. In all animals tested, the increase in arterial pressure in response to the highest three concentrations of vasopressin was greater following cyclooxygenase inhibition than before, while the saline vehicle had no effect. The baroreceptor-mediated bradycardia accompanying the rise in blood pressure was variable, but unaffected by meclofenamate or ibuprofen. It is concluded that vasodilator prostaglandins are released in response to pressor levels of vasopressin, which act to modulate the pressor response of the peptide.

Considerable evidence has accumulated in recent years supporting an important role for vasopressin as a pressor substance in a variety of pathophysiological settings such as DOCA–salt hypertension (1, 2), hemorrhage (3), and water deprivation (4, 5, 6). Recent *in vitro* experiments have shown, however, that vasodilator prostaglandins may be released by vascular smooth muscle cells in response to exogenously applied vasopressin (7). This finding would suggest that the vasoconstrictor effects of vasopressin may be modulated by local prostaglandin release. This hypothesis is supported by data from humans, documenting that the return of total peripheral resistance to normal following vasopressin administration is delayed by pretreatment with the cyclooxygenase inhibitor indomethacin (8). In addition, studies on anesthetized animals have demonstrated enhanced renal vasoconstriction in response to exogenous vasopressin following prostaglandin inhibition (9, 10). However, the physiological significance of these latter findings is uncertain due to the known influence of anesthesia and acute surgical preparation to stimulate endogenous vasopressin and prostaglandin release (11, 12) and thus alter vascular responsiveness to these compounds.

Therefore, the purpose of the present study was to further investigate whether endogenous vasodilator cyclooxygenase products modulate the vasoconstrictor effect of vasopressin *in vivo*. To examine this question in the most physiological setting, experiments were performed on conscious, unstressed rats administered pressor levels of vasopressin with and without cyclooxygenase inhibition.

Methods. All experiments were performed on male Sprague–Dawley rats (body wt = 353 ± 9 g). The animals were initially anesthetized with a combination of ketamine (30 mg/kg im) and sodium pentobarbital (30 mg/kg ip). Chronic arterial and venous polyethylene catheters were aseptically implanted into the right carotid artery and right external jugular vein, respectively. The catheters were routed subcutaneously to the dorsal neck region and exteriorized. The catheters were coiled and placed in a protective plastic container which was sutured to the skin. Both catheters were filled with heparinized saline (50 units/ml) and tied off. The animals were allowed 1–5 days to recover from this surgical procedure. All animals were in good health following surgery as evidenced by normal food and water intake and weight gain.

At the time of study, the rats were placed in a Plexiglas chamber of sufficient size (25 × 10 × 10 cm) to allow free movement, however, small enough to discourage excessive exploration. The bottom of the chamber was covered with fresh bedding for the comfort of the animal, and fresh air was flushed through the chamber continuously. The catheters were

0037-9727/85 $1.50

opened and flushed with approximately 0.1 ml sterile heparinized saline. The venous catheter was connected to a three-way sterile stopcock for intravenous administration of test solutions. The arterial catheter was connected to a Statham P23 DB blood pressure transducer. Blood pressure was recorded with a Grass Model 7 polygraph. Mean arterial blood pressure (MABP) was determined by electronic low-pass filtering of the pulsatile signal. The animals were allowed at least 30 min to equilibrate before any experimental measurements were taken. In all cases, animals were calm and demonstrated stable blood pressure and heart rate.

Group 1—Blood pressure responses to AVP before and after meclofenamate. MABP and heart rate were determined just prior to and following the bolus injection of synthetic arginine vasopressin (AVP) (Pitressin, Parke-Davis) at several doses. Doses examined were 1 mU (n = 6), 2.5 mU (n = 6), 5.0 mU (n = 6), and 12.5 mU (n = 8) (1 mU = 2.5 ng) each in a volume of 100 microliters. Thirty minutes were allowed between AVP injections in experiments where multiple doses were administered in the same animal. In general, MABP and heart rate had returned to control within 10 min of each injection. The maximal increase in mean arterial blood pressure was noted for each level of AVP and the heart rate determined approximately 1 min later. In experiments where multiple doses were administered, the order of injections was randomized.

Following determination of these control responses, the cyclooxygenase inhibitor sodium meclofenamate (3 mg/kg iv) was administered at a dose previously shown to inhibit prostaglandin release in conscious animals (13). Thirty minutes after this injection, the responses to the various concentrations of AVP were determined as before. The order of AVP infusions was again randomized.

Group 2—Blood pressure responses to AVP before and after ibuprofen. Experiments were performed on five additional rats testing the response to all four levels of AVP before and after administration of the structurally dissimilar cyclooxygenase inhibitor ibuprofen at an effective blocking dose (10 mg/kg, iv) (14). Again the order of the AVP injections was randomized and all aspects of these experiments were identical to Group 1.

Group 3—Blood pressure responses to AVP before and after vehicle administration. Responses to the four concentrations of AVP were determined in another group of six rats, both before and after the administration of the saline vehicle for the two cyclooxygenase inhibitors. The time course for these experiments was identical to that of the meclofenamate and ibuprofen groups and thus served as the time control for the other two groups.

Statistical analysis. The change in MABP, HR, and the duration of the pressor response for each concentration of AVP was compared before and after cyclooxygenase inhibition or vehicle administration in each animal on a paired basis utilizing Student's t test. In addition, basal MABP and HR were compared before and after inhibition to test for any change in baseline. Differences were considered significant when $P < 0.05$.

Results. Administration of either cyclooxygenase inhibitor had no consistent effect on basal MABP or HR in the conscious rat. Basal blood pressure before prostaglandin synthesis inhibition was 102.7 ± 7.2 mm Hg compared to 100.6 ± 6.6 mm Hg following meclofenamate in all experiments. Blood pressure was also unchanged in the five rats receiving ibuprofen, however, there appeared to be a tendency for MABP to fall (102.6 ± 6.8 to 96.0 ± 6.7 mm Hg). In a similar manner, MABP was unaffected by saline vehicle administration in the time control (104.8 ± 4.9 vs 105.5 ± 6.7 mm Hg). Basal HR was 376 ± 16 bpm prior to meclofenamate and remained unchanged (381 ± 19 bpm) following inhibitor administration. In addition, HR did not change in response to ibuprofen in the five rats tested (334 ± 18.5 to 350.4 ± 15.7 bpm), or following saline vehicle administration (391.5 ± 15.7 vs 377.4 ± 14.2 bpm).

While basal blood pressure was unaffected by cyclooxygenase inhibition, the increase in MABP resulting from exogenous AVP administration was greater following either meclofenamate (Fig. 1) or the structurally dissimilar inhibitor ibuprofen (Fig. 2). The pressor response to 2.5 mU, 5.0 mU, and 12.5 mU AVP was greater following meclofenamate in all animals tested, while in the five rats given ibuprofen, the blood pressure response to all four AVP levels tested was greater after drug administration. There was considerable variability in the pressor response to a given dose

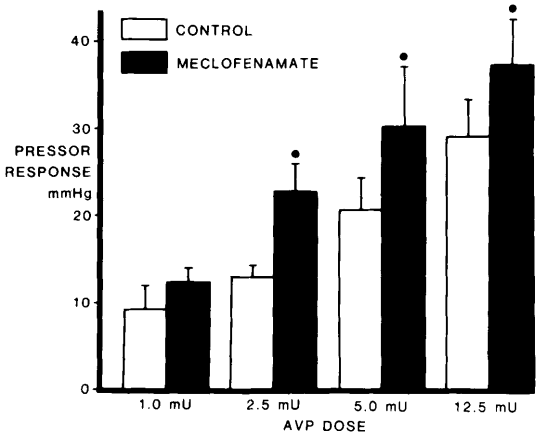

FIG. 1. Arterial blood pressure responses to vasopressin before and after meclofenamate administration. ● Indicates significant difference ($P < 0.05$). Data are $\bar{X} \pm$ SE. ($n = 6$ for 1, 2.5, and 5 mU doses; $n = 8$ for 12.5 mU).

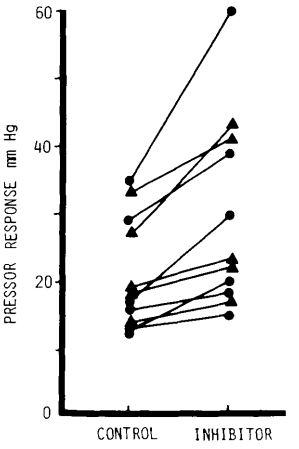

FIG. 3. Pressor responses of individual animals to 5.0 mU AVP before and after cyclooxygenase inhibition with either meclofenamate (circles) or ibuprofen (triangles).

of AVP between rats, however, the response was consistently increased following cyclooxygenase inhibition. Figure 3 presents the pressor responses to 5.0 mU AVP of individual animals before and after cyclooxygenase blockade to illustrate this point. Rats given only the saline vehicle, showed no change in MABP response to AVP. In addition to an increased magnitude of the pressor response to AVP following cyclooxygenase inhibition, the duration of the response was also increased after either meclofenamate or ibuprofen, but not after vehicle treatment (Table I).

The HR response to exogenous AVP was quite variable, especially at the higher doses. However, no consistent effect following meclofenamate, ibuprofen, or saline vehicle administration was noted, except for a slightly greater HR response to 12.5 mU AVP following ibuprofen and to 2.5 mU AVP following vehicle. The heart rate responses of all groups are presented in Table II.

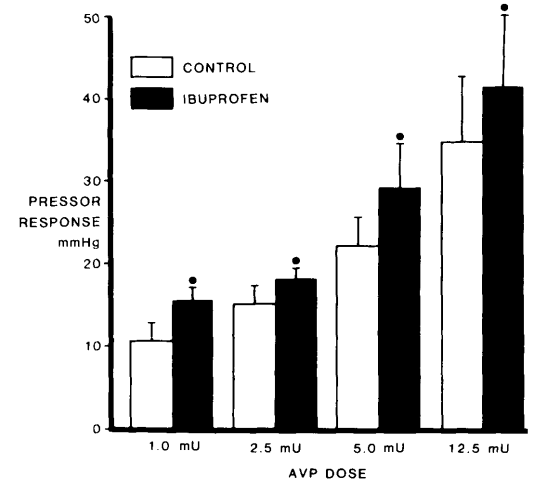

FIG. 2. Arterial blood pressure responses to vasopressin before and after ibuprofen administration in five rats. ● Indicates significant difference ($P < 0.05$). Data are $\bar{X} \pm$ SE.

Discussion. The present study provides *in vivo* evidence that vasodilator cyclooxygenase products are released which modulate the pressor effects of exogenously administered vasopressin. This conclusion is based upon the similar findings following administration of two structurally dissimilar cyclooxygenase inhibitors in conscious, unstressed rats. Both inhibitors elicited an increase in the magnitude and duration of the pressor response to bolus AVP. The rise in blood pressure was partially offset by baroreceptor-mediated bradycardia and a probable decrease in cardiac output, however, cyclooxygenase inhibition did not appear to affect this reflex. Therefore, the observed enhancement of the blood pressure response to vasopressin following prostaglandin synthesis inhibition is likely due to prevention of the local release of vasodilator prostaglandins.

Recent *in vitro* experiments have demonstrated that vasopressin stimulates the production of prostacyclin (PGI_2), a potent va-

TABLE I. DURATION (min) OF PRESSOR RESPONSE TO DIFFERENT DOSES OF VASOPRESSIN (AVP)

	1.0 mU AVP	2.5 mU AVP	5.0 mU AVP	12.5 mU AVP
Control	1.2 ± 0.3	2.5 ± 0.3	4.2 ± 0.8	8.1 ± 1.4
	(n = 6)	(n = 6)	(n = 6)	(n = 8)
Meclofenamate	3.0 ± 0.4*	5.4 ± 1.5*	7.4 ± 1.2*	11.2 ± 2.2*
Control	1.5 ± 0.5	3.0 ± 0.4	5.4 ± 1.1	6.6 ± 1.2
	(n = 5)	(n = 5)	(n = 5)	(n = 5)
Ibuprofen	3.2 ± 0.8*	4.5 ± 0.8*	7.6 ± 1.6	11.8 ± 2.3*
Control	2.1 ± 0.3	2.8 ± 0.6	4.2 ± 0.5	10.1 ± 3.8
	(n = 6)	(n = 6)	(n = 6)	(n = 6)
Vehicle	1.4 ± 0.6	2.6 ± 0.7	4.0 ± 0.9	9.4 ± 3.2

Note. Data are $\bar{X}$ ± SE. *Indicates significantly different from control ($P < 0.05$).

sodilator, by cultured vascular smooth muscle (7). These experiments on smooth muscle cells cultured from rat mesenteric arteries also documented enhanced PGI_2 synthesis in response to angiotensin II, however, the maximal PGI_2 release was considerably greater in response to vasopressin. The vasoconstrictor activity of vasopressin seemed important to the stimulation of PGI_2 release from these smooth muscle cells, since a vasopressin analog without pressor properties was much less effective. In addition, other investigators have shown that increases in vasopressin concentration also appear to stimulate renal prostaglandin production in a number of species including the rat where PGE_2 release may be elevated (15, 16, 17). Cooper and Malik (18) have recently shown that vasopressin stimulates PGI_2 release from the isolated rat kidney as well. Similar to the findings in cultured vascular smooth muscle, these investigators also reported that the binding of vasopressin receptors by a structural vasopressin analog without pressor properties does not release PGI_2, however, PGI_2 release was unchanged when the AVP pressor effect was blocked by calcium removal. In addition, other *in vivo* observations in anesthetized rats indicate that cyclooxygenase inhibition causes an increased renal vasoconstriction in response to exogenous vasopressin (9, 10). Since Liard *et al.* (19) have reported that after infusion of vasopressin for 1 h in conscious dogs renal blood flow is unaltered, a physiological role for PGI_2 as a vasodilator maintaining renal blood flow under these conditions is attractive.

In addition to these experiments on specific vascular beds, Glänzer *et al.* (8) have reported that pretreatment of human volunteers with indomethacin prevented the normal return to baseline of total peripheral resistance during continued vasopressin infusion. These data

TABLE II. CHANGE IN HEART RATE IN RESPONSE TO DIFFERENT DOSES OF VASOPRESSIN (AVP)

	1.0 mU AVP	2.5 mU AVP	5.0 mU AVP	12.5 mU AVP
Control	−18.0 ± 4.5	−39.3 ± 10.7	−25.3 ± 6.7	−55.5 ± 13.6
	(n = 6)	(n = 6)	(n = 6)	(n = 8)
Meclofenamate	−22.7 ± 8.1	−28.0 ± 10.2	−24.7 ± 3.0	−66.5 ± 12.0
Control	−10.4 ± 5.6	−22.4 ± 8.6	−17.6 ± 8.2	−36.0 ± 6.8
	(n = 5)	(n = 5)	(n = 5)	(n = 5)
Ibuprofen	−17.6 ± 5.6	−27.2 ± 8.1	−30.4 ± 6.9	−55.2 ± 8.4*
Control	−18.0 ± 4.2	−10.7 ± 6.4	−44.7 ± 9.0	−51.7 ± 19.5
	(n = 6)	(n = 6)	(n = 6)	(n = 6)
Vehicle	−14.7 ± 7.6*	−27.3 ± 5.6	−34.7 ± 7.3	−59.3 ± 12.5

Note. Data are $\bar{X}$ ± SE. *Indicates significantly different from control ($P < 0.05$).

suggest that endogenous vasodilator prostaglandins are responsible for the tachyphylaxis normally observed after several minutes of constant vasopressin infusion. In contrast to the present findings however, the peak pressor response to vasopressin was unaltered. This apparent discrepancy is likely related to the different modes of administration used in the two studies, since a slow infusion of vasopressin permits the parallel activation of the baroreceptor reflex, while the peak response to a bolus of vasopressin largely precedes such buffering effects. Both studies demonstrate an increased duration of the pressor response, however. Therefore, vasodilator prostaglandins may be of importance in both short- and long-term modulation of the pressor effects of vasopressin.

The release of vasodilator prostaglandins in response to a variety of pressor stimuli has been suggested by other investigators. For example, in a recent study by Inokuchi and Malik (20), meclofenamate was shown to augment renal vasoconstriction in response to either renal nerve stimulation or norepinephrine injection in anesthetized rats. In addition, prostaglandin synthesis inhibition has also been shown to potentiate vasoconstriction in mesenteric arteries following angiotensin II administration (21) as well as to prevent subsequent relaxation in the same preparation (21, 22). Elevated PGI_2 release has also been documented in the lung in response to pulmonary vasoconstriction (23). It therefore appears that vasodilator prostaglandins are important modulators of a number of vasoconstrictor stimuli in several vascular beds. The present findings suggest that vasodilator cyclooxygenase products are released in response to vasopressin-induced vasoconstriction as well.

Conclusion. The prior administration of two structurally dissimilar cyclooxygenase inhibitors, meclofenamate or ibuprofen, results in a potentiated increase in blood pressure following bolus vasopressin administration in conscious rats. These findings and *in vitro* evidence (7) suggest that vasodilator prostaglandins are released during vasopressin-induced vasoconstriction, and act to modulate the pressor effects of this peptide.

I thank Janice Walker for technical support in these studies and Glenda Bailey and Myrna Romain for secretarial assistance. This work was supported by a grant from the American Heart Association of Louisiana, Inc.

1. Crofton JT, Share L, Shade RE, Lee-Kwon WJ, Manning M, Sawyer WH. The importance of vasopressin in the development and maintenance of DOC-salt hypertension in the rat. Hypertension **1**:31–38, 1979.
2. Berecek KH, Murray RD, Gross F, Brody MJ. Vasopressin and vascular reactivity in the development of DOCA hypertension in rats with hereditary diabetes insipidus. Hypertension **4**:3–12, 1982.
3. Schwartz J, Reid IA. Effect of vasopressin blockade on blood pressure regulation during hemorrhage in conscious dogs. Endocrinology **109**:1778–1780, 1981.
4. Aisenbrey GA, Handelman WA, Arnold P, Manning M, Schrier RW. Vascular effects of arginine vasopressin during fluid deprivation in the rat. J Clin Invest **67**:961–968, 1981.
5. Andrews CE, Brenner BM. Relative contributions of arginine vasopressin and angiotensin II to maintenance of systemic arterial pressure in the anesthetized water-deprived rat. Circ Res **48**:254–258, 1981.
6. Schwartz J, Reid IA. Role of vasopressin in blood pressure regulation in conscious water-deprived dogs. Amer J Physiol **244**:R74–R77, 1983.
7. Hassid A, Williams C. Vasoconstrictor-evoked prostaglandin synthesis in cultured vascular smooth muscle. Amer J Physiol **245**:C278–C282, 1983.
8. Glänzer K, Prussing B, Dusing R, Kramer HJ. Hemodynamic and hormonal responses to 8-arginine-vasopressin in healthy man: Effects of indomethacin. Klin Wochenschr **60**:1234–1239, 1982.
9. Oliver JA, Sciacca RR, Le Cren G, Cannon PJ. Modulation by prostaglandins of the renal vascular action of arginine vasopressin. Prostaglandins **24**:641–656, 1982.
10. Hofbauer KG, Dienemann H, Forgiarini P, Stalder R, Wood JM. Renal vascular effects of angiotensin II, arginine-vasopressin and bradytkinin in rats: Interactions with prostaglandins. Gen Pharmacol **14**:144–147, 1983.
11. Bonjour JP, Malvin RL. Plasma concentration of ADH in conscious and anesthetized dogs. Amer J Physiol **218**:1128–1132, 1970.
12. Terragno NA, Terragno DA, McGiff JC. Contribution of prostaglandins to the renal circulation in conscious, anesthetized, and laparotomized dogs. Circ Res **40**: 590–595, 1977.
13. Walker BR. Suppressed basal antidiuretic hormone release during cyclooxygenase inhibition in conscious dogs. Amer J Physiol **244**:R487–R491, 1983.
14. Feigen LP, King LW, Ray J, Beckett W, Kadowitz PJ. Differential effects of ibuprofen and indomethacin in the regional circulation of the dog. J Pharmacol Exp Ther **219**:679–684, 1981.
15. Beck TR, Hassid A, Dunn MJ. The effect of arginine vasopressin and its analogs on the synthesis of prostaglandin E_2 by rat renal medullary interstitial cells in culture. J Pharmacol Exp Ther **215**:15–19, 1980.

16. Scharschmidt L, Dunn MJ. Prostaglandin synthesis by rat glomerular mesangial cells in culture: The effects of angiotensin II and arginine vasopressin. J Clin Invest **71**:1756–1764, 1983.
17. Walker LA, Whorton AR, Smigel M, France R, Frolich JC. Antidiuretic hormone increases renal prostaglandin synthesis in vivo. Amer J Physiol **235**:F180–F185, 1978.
18. Cooper CL, Malik KU. Mechanism of action of vasopressin on prostaglandin synthesis and vascular function in the isolated rat kidney: Effect of calcium antagonists and calmodulin inhibitors. J Pharmacol Exp Ther **229**:139–147, 1984.
19. Liard JF, Deriaz O, Schelling P, Thibonnier M. Cardiac output distribution during vasopressin infusion or dehydration in conscious dogs. Amer J Physiol **243**: H664–H669, 1982.
20. Inokuchi K, Malik KU. Attenuation by prostaglandins of adrenergically induced renal vasoconstriction in anesthetized rats. Amer J Physiol **246**:R228–R235, 1984.
21. Blumberg AL, Denny SE, Marshall GR, Needleman P. Blood vessel–hormone interactions: Angiotensin, bradykinin and prostaglandins. Amer J Physiol **232**: H305–H310, 1977.
22. Webb RC. Angiotensin II-induced relaxation of vascular smooth muscle. Blood Vessels **19**:165–176, 1982.
23. Voelkel NF, Gerber JG, McMurtry IF, Nies AS, Reeves JT. Release of vasodilator PGI_2 from isolated rat lung during vasoconstriction. Circ Res **48**:207–213, 1981.

Received January 28, 1985. P.S.E.B.M. 1985, Vol. 180.
Accepted May 29, 1985.