Role of AVP in pressor responses during activation of central TxA2/PGH2 receptors

CHRISTOPHER S. WILCOX, HUBIN GAO, JOSEPH G. VERBALIS, AND WILLIAM J. WELCH
Division of Nephrology and Hypertension and Division of Endocrinology and Metabolism, Georgetown University Medical Center, Washington, District of Columbia 20007

Wilcox, Christopher S., Hubin Gao, Joseph G. Verbalis, and William J. Welch. Role of AVP in pressor responses during activation of central TxA2/PGH2 receptors. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1927–H1932, 1997.—Administration of thromboxane A2/prostaglandin H2 (TxA2/PGH2)-receptor agonist U-46619 (2.86 nmol/kg iv) to conscious rats increased mean arterial pressure (MAP) by 17 ± 2 mmHg (n = 6; P < 0.001) and plasma arginine vasopressin (AVP) by 3.5 ± 1.1 IU/ml (n = 6; P < 0.001). Ifetroban (TxA2/PGH2 antagonist; intracerebroventricularly) prevented both responses. Intracerebroventricular U-46619 increased MAP in Long-Evans rats (n = 6) more than in AVP-deficient Brattleboro rats. AVP V1-receptor antagonist d(CH2)5Tyr(Me)AVP (3 µg/kg iv) blocked 67% and 69% of pressor response to intravenous AVP and intracerebroventricular U-46619, respectively. AVP (10 ng/kg iv) increased AVP by 4.7 ± 0.5 pg/ml, comparable to the increase of 3.5 ± 1.2 pg/ml with intracerebroventricular U-46619 (2.86 nmol/kg), but the rise in MAP was only one-half as great (+8 ± 3 mmHg for AVP vs. +17 ± 2 mmHg for U-46619; P < 0.05). In conclusion, U-46619 raises blood pressure and releases AVP by activating brain receptors. AVP explains approximately one-half of the pressor response.

Vasopressin; thromboxane A2/prostaglandin H2 receptors; ifetroban; brain; U-46619

THROMBOXANE A2 (TxA2), as assessed from measurements of its metabolite, TxB2, is generated in rat (23) and cat (25) brain and is released from isolated guinea pig hypothalamo-neurohypophysis explants (12). Messenger RNA encoding TxA2/prostaglandin (PG) H2 receptors is expressed in rat neuronal and astroglial cells in culture (8, 9, 14) and rat brain stem (9). However, the function of these receptors is poorly understood. Central administration of a TxA2/PGH2 receptor agonist, U-46619, increases mean arterial pressure (MAP) in conscious, spontaneously hypertensive rats (SHR) (24) and normal Sprague-Dawley rats (9). Because prostaglandins (PG) can regulate arginine vasopressin (AVP) secretion (5, 6, 12, 30) and release of AVP contributes to the pressor response to central administration of angiotensin II (ANG II) (30), the present studies were designed to investigate the hypothesis that activation of central TxA2/PGH2 receptors releases AVP and that this contributes to the pressor response.

To test this hypothesis, U-46619 was injected directly into the lateral cerebral ventricle of conscious, unrestrained Sprague-Dawley rats and the MAP and plasma AVP responses were established. The role of central TxA2/PGH2 receptors was investigated in groups of rats given the specific antagonist ifetroban (21) intracerebroventricularly. The contribution of AVP to the pressor response was assessed from three protocols. First, groups of Sprague-Dawley rats were pretreated intravenously with the AVP V1-receptor antagonist d(CH2)5Tyr(Me)AVP. Second, the pressor responses to intracerebroventricular U-46619 were contrasted in control Long-Evans and AVP-deficient Brattleboro rats. Third, the rise in MAP produced by intracerebroventricular U-46619 was compared with that produced from an intravenous infusion of AVP concentration that raised the plasma AVP to a similar extent.

METHODS

Animal Preparation

Studies were undertaken in male Sprague-Dawley rats weighing 175–275 g. Rats were fed a normal salt diet (Rat Chow; Ralston Purina, St. Louis, MO) with a sodium content of 0.3 g/100 g. Rats were allowed free access to food and water. All studies were undertaken in conscious, unrestrained rats habituated to their cages, as described previously (9, 28). Rats were anesthetized with pentobarbital sodium (50 mg/kg ip; Abbott Labs, North Chicago, IL). A PE-10 catheter, connected to a PE-50 catheter, was placed in the femoral artery, and a PE-50 catheter was placed in the femoral vein. Catheters were threaded subcutaneously to the nape of the neck, where they exited the cage via a light, flexible metal spring to provide protection. This was secured on one end to a plate that was sutured to the skin of the rat and on the other end to a swivel that allowed the rat to turn in the cage. Catheters were flushed and filled with a solution of 500 IU/ml of heparin and 1,000 IU/ml of penicillin G in 0.154 M saline. Two days later, rats that required intracerebroventricular injections were anesthetized with pentobarbital sodium. With the use of a stereotaxic frame (David Kopf, Tujunga, CA), the skull was immobilized and drilled, and a 23-gauge stainless steel guide cannula was lowered 5 mm into the lateral cerebral ventricle, which was located 1.4 mm lateral and 0.9 mm posterior to the bregma. After two stainless steel anchoring screws were inserted, dental acrylic was applied to secure the guide cannula to the skull and sealed with a 30-gauge stainless steel obturator. After recovering from anesthesia, rats were placed in individual metabolism cages with free access to food and water. Catheters were flushed daily.

Rats were allowed 3 days to recover from surgery. The arterial line was connected to a pressure transducer to record the MAP and heart rate (HR) on a computer after analog-to-digital conversion (MacLab 4E; AD Instruments, Milford, MA). For intracerebroventricular injections, the obturator of the intracerebral cannula was removed and replaced with an injector inserted via the guide cannula and connected to a microsyringe via a PE-20 catheter. The system was filled with test solution, and the desired volume (10 µl) was injected when required. The position of the intracerebroventricular cannula was confirmed at the end of the experiments by injection of methylene blue and postmortem examination of the brain.
Experimental Protocols

The aim of series 1 was to assess AVP release after activation of central TxA2/PGH2 receptors. Rats (n = 6) were prepared with intravenous, intra-atrial, and intracerebroventricular cannulas. A sample of arterial blood (500 µl) was withdrawn and replaced with an equivalent volume of 6% albumin (bovine serum albumin; Sigma, St. Louis, MO) in 0.154 M saline. Thereafter, 15 min was allowed, followed by 30 min of recording of MAP and HR. On one occasion, vehicle (0.154 M NaCl solution) was injected intracerebroventricularly 1 min after U-46619 (1 µg/kg or 2.86 nmol/kg), and blood pressure and HR were recorded 1 min later, followed by withdrawal of a blood sample (500 µl, replaced by albumin saline). This time interval corresponds to the maximum rise in blood pressure (9). On another occasion, separated from the first by 24–48 h, rats were restudied (order randomized). The TxA2/PGH2-receptor antagonist ifetroban (0.1 µg/kg or 138 pmol/kg) (21) was given intracerebroventricularly during the basal blood sampling and 1 min before intracerebroventricular administration of U-46619. The order of studies (vehicle or ifetroban) was randomized among rats.

The aim of series 2 was to contrast the pressor responses to intracerebroventricular administration of U-46619 in control Long-Evans rats (n = 6) and AVP-deficient Brattleboro rats (n = 6). Rats were prepared as in series 1 with intracerebroventricular cannulas. Previous studies (9) have shown that doses of U-46619 of 0.3, 1, and 3 µg/kg [0.86, 2.86, and 8.6 nmol/kg intracerebroventricularly (ivc)] at 20-min intervals produced graded increases in MAP in conscious Sprague-Dawley rats. This protocol was repeated in series 2. The maximal pressor responses were seen 1 min after intracerebroventricular drug administration, at which time MAP and HR were recorded.

The aim of series 3 was to assess the efficacy of an AVP V1-receptor antagonist in blocking pressor responses after intracerebroventricular administration of U-46619. Rats (n = 6) were prepared as in series 1 and studied on 2 days, separated by 24 h, and in random order. On 1 day, MAP (0, 10, and 40 ng/kg iv) was administered. These doses were selected from pilot studies to produce rises in MAP that were comparable to those produced by the intracerebroventricular administration of U-46619. The order of studies (vehicle or ifetroban) was randomized among rats.

The aim of series 4 was to compare the relationship between MAP and plasma AVP concentration after intracerebroventricular U-46619 or intravenous AVP. These data were used to determine the extent to which the rise in MAP with intracerebroventricular U-46619 could be ascribed to circulating AVP. Rats (n = 4) were prepared with intravenous and intra-atrial cannulas. The schedule of AVP injections at 0, 10, and 40 ng/kg given intravenously in series 3 was used in this separate group of rats. Blood samples (500 µl, replaced with 6% albumin in saline) were taken 1 min after each injection, corresponding to the time of peak pressor response. The plasma was separated and subsequently analyzed for AVP concentration.

Statistical Analysis

Data were assessed by analysis of variance with repeated measures. Where appropriate, Dunnett’s t-test was applied to detect differences between groups. Data are means ± SE, and statistical significance was taken at P < 0.05.

RESULTS

The MAP and HR of the conscious rats averaged 107 ± 3 mmHg and 409 ± 5 beats/min, respectively.

Series 1

The intracerebroventricular administration of U-46619 (1 µg/kg or 2.86 nmol/kg) increased MAP by 17 ± 2 mmHg. As described previously (9), the MAP increased to a maximal level at 1 min, followed by a decline over 30 min to a value close to baseline. This was significantly blunted by intracerebroventricular administration of ifetroban (0.1 µg/kg or 138 pmol/kg) (Fig. 1). Plasma AVP concentration averaged 2.3 ± 0.2 pg/ml after intracerebroventricular administration of vehicle. After intracerebroventricular administration of U-46619, plasma levels of AVP more than doubled to 5.8 ± 1.0 pg/ml (Fig. 2A). After intracerebroventricular administration of ifetroban, basal plasma levels of AVP were not different from vehicle and averaged 2.2 ± 0.2 pg/ml. After ifetroban was administered, plasma AVP concentration was significantly blunted.

Drugs. U-46619 (9,11-dideoxy 11α,9α-epoxymethanoprostaglandin F2α; mol wt 350) was a gift from Upjohn Pharmaceuticals (Kalamazoo, MI). It was shipped in methyl acetate and stored at −20°C. Stock solutions were prepared by addition of tris(hydroxymethyl)methane (Fisher Scientific, Fair Lawn, NJ), dried in a stream of air, dissolved in 0.154 M NaCl, and stored at −20°C. The dilutions were made freshly in 0.154 M NaCl before each experiment. Ifetroban (BMS-180291; mol wt 724) was a gift from Martin Ogletree of Bristol-Myers Squibb (Princeton, NJ). It was dissolved freshly in 0.154 M NaCl before each experiment. AVP was obtained from Sigma, and d(CH2)5Tyr(Me)AVP [1-ß-mercapto-ß-cyclopentamethylene propionic acid], 2-(O-methyl)L-lysine]-Arg-8-vasopressin (15) was obtained from Peninsula Laboratories (Belmont, CA). Both were dissolved in 0.154 M saline immediately before use.
was not significantly changed by intracerebroventricular administration of U-46619 and averaged 2.4 ± 0.4 pg/ml (Fig. 2B).

**Series 2**

Intracerebroventricular administration of U-46619 caused graded increases in MAP in Long-Evans rats (Fig. 3), although the increase in MAP after 1 µg/kg (2.86 nmol/kg) was not as great as in series 1 in Sprague-Dawley rats. Similar intracerebroventricular administration of U-46619 to AVP-deficient Brattleboro rats produced much smaller increases in MAP (Fig. 3).

**Series 3**

Intravenous administration of AVP (40 ng/kg) raised MAP by 17 ± 1 mmHg. This effect was blunted 67% by prior intravenous administration of the AVP V1-receptor antagonist d(CH2)5Tyr(Me)AVP (Fig. 4). As in series 1, the intracerebroventricular administration of U-46619 (1 µg/kg or 2.86 nmol/kg) raised MAP by 17 ± 2 mmHg. Prior intravenous administration of d(CH2)5Tyr(Me)AVP blunted ~69% of this pressor response.
Plasma levels of AVP increased with bolus intravenous injections of AVP. Intravenous administration of AVP (40 ng/kg) raised MAP by 19 ± 7 mmHg (n = 6), which was comparable to the rise of 17 ± 2 mmHg produced by intracerebroventricular administration of U-46619 (1 µg/kg or 2.86 nmol/kg). However, as shown in Fig. 5, the increase in plasma AVP concentration was threefold greater after intravenous AVP than after intracerebroventricular U-46619.

Intravenous administration of AVP (10 ng/kg) raised plasma AVP concentration by 4.7 ± 0.5 pg/ml (n = 4), which was comparable to the rise of 3.5 ± 1.2 pg/ml (n = 6) produced by intracerebroventricular administration of U-46619 (1 µg/kg or 2.86 nmol/kg). However, as shown in Fig. 6, the increase in MAP was twice as great after intracerebroventricular U-46619 than after intravenous AVP. These data indicate that the increase in plasma AVP concentration after intracerebroventricular administration of U-46619 can potentially explain only approximately one-half of the observed rise in MAP after intracerebroventricular administration of U-46619.

**DISCUSSION**

The main new findings of this study are that central administration of a TxA2/PGH2-receptor agonist increases plasma AVP concentrations, and multiple lines of evidence indicate that the AVP release contributes to the observed pressor responses to this agent: 1) blockade of AVP V1 receptors blunts the pressor response, 2) the pressor response is diminished in AVP-deficient Brattleboro rats, and 3) a dose of intravenous AVP that raised the plasma AVP level to that produced by intracerebroventricular U-46619 increased MAP by about one-half as much.

Several recent studies have provided evidence for central TxA2/PGH2 receptors, although their function is presently not well understood. Rat neuronal (8, 9) and astroglial (9, 14) cells in culture and rat brain stem (9) contain mRNA for TxA2/PGH2 receptors corresponding to that from rat kidney (1). TxA2/PGH2 binding sites linked to activation of phospholipase C have been detected in human (20) and rabbit (19) astrocytes. TxA2 can be formed at several sites within the brain. Astroglial cells and, to a lesser extent, neuronal cells synthesize TxA2 (22), as do cerebral arterioles and capillaries (17) and rat brain (16). TxA2 is released from the brain and spinal cord during cerebral hypoxia (3, 23).

The hypothalamus regulates AVP release from the posterior pituitary. Two studies have identified this region as a site for TxA2 generation. Ex vivo studies have shown that TxA2 is generated in the isolated hypothalamo-neurohypophysial explant of the guinea pig (12). In vivo studies in the cat, with the use of a “push-pull” perfusion system, have shown that PGE2 and TxB2 are released from the preoptic, anterior hypothalamic and tuberal-posterior hypothalamic regions of conscious cats (25). In the basal state, release of TxB2 is greater than that of PGE2 and is increased 2- to 10-fold by local application of lipopolysaccharide. Collectively, these studies indicate that the brain has substantial capacity for generation of TxA2, especially in the regions implicated in AVP regulation. Moreover, there are other endogenous ligands for the TxA2/PGH2 receptor that include endoperoxides and isoprostanes (29).

The observation that central administration of U-46619 increases blood pressure in the conscious rat...
confirms a previous study (9) in which this central pressor response was more fully characterized. The response was found to be dose dependent and inhibited by ifetroban. A previous study (24) also observed central pressor responses with U-46619 in conscious SHR but did not detect such responses in normotensive Wistar rats. These differences suggest that there may be differences among rat strains in the central TXA2/PGH2 pressor pathway.

Several lines of evidence have implicated AVP release in the pressor responses of several central treatments. Ventriculocisternal perfusion of ANG II in the dog (30) or intracerebroventricular injections of ANG II in the rat (10) release AVP, which contributes to the central pressor pathway. Similarly, microinjection of endothelin-1 into the subfornical organ of the rat increases blood pressure and doubles plasma levels of AVP (18). The pressor response is blunted by V1 receptor antagonists, suggesting that it depends largely on AVP release. Although intracarotid infusions of PGE2 (7) or U-46619 (27) in sheep do not alter AVP release, other studies have implicated cerebral PG in the regulation of AVP secretion. Thus intracerebroventricular administration of PGE2 in the sheep (4) or rat (11) increases AVP release. PGE2, as well as ANG II, stimulates AVP release from ex vivo hypothalamic-neurohypophyseal explant of the guinea pig (2). Both in vivo (30) and in vitro (2) studies implicate PG in the AVP response to central ANG II. Furthermore, cyclooxygenase inhibitors can blunt or prevent AVP release in response to dehydration or hemorrhage (5). Thus hypothalamic PG may be important mediators of AVP release, in both the response to stimulation by pressor systems such as ANG II and the regulation of AVP secretion by osmotic or volume-dependent mechanisms. The present findings suggest that TXA2/PGH2 receptors could be involved in these regulatory processes, but this remains to be tested.

The present study provides what we believe to be the first evidence for stimulation of AVP release after activation of central TXA2/PGH2 receptors. We found that plasma AVP concentrations increased more than twofold 1 min after intracerebroventricular injection of U-46619. This is likely a response to the TXA2 mimetic rather than to the rise in blood pressure, because AVP release is stimulated by reductions, rather than increases, in blood pressure (5). Two distinct lines of evidence implicate AVP release in the pressor response to central administration of U-46619. First, blockade of AVP V1 receptors with d(CH2)5Tyr(Me)AVP blocked >65% of the increase in blood pressure. Second, comparison of the dose-response relationships for central U-46619 in AVP-deficient Brattleboro and control Long-Evans rats, which are of the same rat strain, demonstrated that, in the absence of AVP, the rise in MAP was reduced to about one-third. Nevertheless, there was still some increase in MAP after intracerebroventricular U-46619 in Sprague-Dawley rats given d(CH2)5Tyr(Me)AVP and in Brattleboro rats. The data therefore indicate the activation of pressor pathways in addition to AVP. To assess the quantitative importance of increases in plasma AVP to the rise in blood pressure with central administration of U-46619, we compared changes in blood pressure and plasma AVP during central U-46619 with those after AVP injection. We found that a dose of intravenous AVP that raised the blood pressure to the same extent as central U-46619 increased the plasma AVP concentration threefold more than that of central U-46619. This indicates that not all the increase in blood pressure with central U-46619 can be ascribed to increases in plasma AVP concentration. Moreover, an intravenous injection of AVP that raised the plasma concentration of AVP to the same extent as central U-46619 raised the blood pressure by only one-half as much. Collectively, these data indicate that approximately one-half of the pressor response to central U-46619 can be ascribed to release of AVP into the peripheral circulation. In a previous study, we compared plasma 3H activity after intracerebroventricular and intravenous administration of [3H]U-46619 (9). One minute after intracerebroventricular injection, there was already plasma 3H detectable, although only 12% of that was detectable after an intravenous injection of an equivalent dose. This suggests that some of the pressor response to intracerebroventricular U-46619 that could not be accounted for by AVP release may be secondary to systemic effects of U-46619.

In summary, these studies demonstrate a novel pressor pathway activated by central TXA2/PGH2 receptors which is mediated, in part, by pituitary AVP release. The physiological importance of this pathway for blood pressure and body fluid homeostasis remains to be investigated.

We are grateful to Drs. Karen Gayle and David Dybdal for assistance in the technique of central placement of intracerebroventricular cannulas in the rat and Drs. Richard A. Gillis and J oe A. DiMico for helpful discussion. U-46619 was kindly provided by Upjohn Pharmaceuticals (Kalamazoo, MI), and ifetroban was provided by Martin Oglee of Bristol-Myers Squibb (Princeton, NJ ). We are grateful to Marly Davidson for preparing the manuscript.

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-38994, DK-36079, and DK-49870 and the George E. Schreiner Chair of Nephrology, H. Gao was supported by an International Society of Nephrology Fellowship Grant.

Address for reprint requests: C. S. Wilcox, Div. of Nephrology and Hypertension, Georgetown Univ. Medical Center, 3800 Reservoir Rd., NW, PHC F6003, Washington, DC 20007.

Received 5 May 1997; accepted in final form 23 June 1997.

REFERENCES


4. Breuhaus, B. A., K. T. Demarest, and J. E. Chimosky. Comparison of intracerebroventricular and intracarotid infu-
H1932

AVP RELEASE BY TXA2/PGH2 RECEPTORS

8. 5.
9. 10.