Vascular Responses to Vasopressin are Tone-Dependent in the Cerebral Circulation of the Newborn Pig

William M. Armstead, Robert Mirro, David W. Busija, and Charles W. Leffler

The effects of lysine vasopressin (LVP) on pial arteriolar diameter and cortical periarachnoid fluid prostanoid concentrations were investigated in newborn pigs. Chloralose-anesthetized piglets were equipped with closed cranial windows over the parietal cortex for observation of pial arterioles and collection of cerebrospinal fluid (CSF) passing over the cerebral surface. Prostanoids in the CSF were determined by radioimmunoassay. LVP (10−1,000 µU/ml) elicited concentration-dependent increases in pial arteriolar diameter associated with increased levels of 6-keto-prostaglandin (PG)F₁₂, PGE₂, thromboxane B₂, and PGF₂α. LVP-induced pial arteriolar dilation was unchanged after intravenous indomethacin (5 mg/kg). Conversely, LVP constricts pial arterioles previously dilated by physiological (hemorrhagic hypotension) and pharmacological (topically applied PGE₂ or isoproterenol) intervention. This constriction is potentiated by indomethacin. Vascular and biochemical changes elicited by LVP were blocked by intravenous [1-(β-mercapto-β-cyclopentamethylene propionic acid),2,(O-methyl)-Tyr-AVP] (5 µg/kg), a putative V₁ receptor antagonist, whereas vascular effects of norepinephrine and U46619, a thromboxane A₂ mimic, were unchanged. Therefore, the degree of vascular tone appears to influence responses of the newborn pig cerebral circulation to LVP. (Circulation Research 1989;64:136-144)

Although vasopressin has profound peripheral hemodynamic effects, its effects on the cerebral circulation are not clear. Immunocytochemical studies suggest that vasopressin may be a neurotransmitter or neuromodulator in the brain, and vasopressin may be found in the cerebrospinal fluid (CSF) where it has access to cerebrovascular smooth muscle. Additionally, the nature of vasopressin receptors present in the cerebral circulation is not clearly defined.

Prostanoids appear to have an important role in the regulation of cerebral hemodynamics in the perinatal period. In previous studies, we found that there is an important interaction between neurotransmitters such as norepinephrine, acetylcholine, and histamine such that these neurotransmitters increase cerebral prostanooid production and that this production influences cerebral vascular responses. Whether prostanoids also are involved in the actions of vasopressin in the cerebral circulation is unclear.

The present study was designed to test the hypothesis that vasopressin has vasoactive effects in the cerebral circulation and that its effects are modulated by prostanoids. In contrast to other species, lysine vasopressin is the form present in the pig. Therefore, we investigated the following parameters in the intact cerebral microcirculation of the newborn pig: 1) the nature of the vascular response to lysine vasopressin (LVP), 2) whether the vascular response to LVP is modulated by prostanoids, and 3) whether LVP-induced changes could be blocked by the putative V₁ (vascular) receptor antagonist [1-(β-mercapto-β-cyclopentamethylene propionic acid),2,(O-methyl)-Tyr-AVP] (MEAVP).

Materials and Methods
Animal procedures were reviewed and approved by the University of Tennessee, Memphis, Animal Care and Use Committee. Thirty-eight piglets (2−6 days old, 0.9−2.1 kg) of either sex were used in these experiments. They were anesthetized with ketamine hydrochloride (33 mg/kg i.m.) and acepromazine (3.3 mg/kg i.m.). Anesthesia was main-
Preparation

Hemorrhagic hypotension was induced by withdrawing blood (about 15 ml/kg) sufficient to reduce mean arterial pressure by 50%. Furthermore, in order to investigate the receptor mechanism of LVP-induced vascular changes, responses to LVP were obtained before and 30 minutes after MEAVP (Bachem Biochemicals, Torrance, California), a putative V1 receptor antagonist (5 μg/kg i.v.). The selectivity of blockade was assessed by obtaining responses to norepinephrine (Sigma) and U46619 (Upjohn) before and 30 minutes after MEAVP, 5 μg/kg i.v.

The LVP stock solution, 1 U/ml was stored at -70°C, and the appropriate dilutions were made on the day of use. The vehicle for the LVP stock was 0.9% NaCl, 0.1% bovine serum albumin, and 0.03% acetic acid. The vehicle for the MEAVP, norepinephrine, and isoproterenol stock solutions was 0.9% NaCl, while the vehicle for the PGE2 and U46619 stock solutions was ethanol. All drug solutions were made fresh on the day of use. The stock solutions for LVP, norepinephrine, isoproterenol, PGE2, and U46619 were diluted with CSF to make the appropriate concentrations, which were applied to the cerebral cortex. The CSF-vehicle for each of these agents had no effect on pial arteriolar diameter.

Protocol

Pial arteriolar diameter was determined following infusion under the window of artificial CSF containing no drug and after infusion of CSF containing 10–1,000 μU/ml of LVP (W.H. Sawyer, Pharmacology Department, Columbia University, 1 μU/ml=4 pg/ml). In the pig, lysine, as opposed to arginine, is the form of vasopressin present. Diameters were also measured 10–15 minutes after flushing out the highest concentration of LVP with CSF containing no drug. Typically, 1–2 ml of CSF were flushed through the window over 30 seconds. Needles incorporated into the side of the window allowed infusion of CSF under the window and runoff of excess CSF. We measured the peak response, which was constant over 1–3 minutes after administration of CSF containing LVP. A CSF sample for prostanoid analysis was collected at the end of a 10-minute collection period. Time control experiments were designed such that responses to LVP were obtained initially and again 30 minutes later.

Statistical Analysis

Data were analyzed using repeated measures analysis of variance, t test, or linear regression.
Table 1. Pial Arteriolar Responses to LVP, Norepinephrine, and U46619 in the Newborn Pig

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<th>Agent</th>
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<td>Normotensive conditions*</td>
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<tr>
<td>LVP (μU/ml)</td>
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<td>159±6</td>
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<td>190±6†</td>
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<td>1,000</td>
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n=5.

*Time control experiments for lysine vasopressin (LVP) under normotensive and hypotensive conditions were conducted in two separate series of animals. Pial arteriolar diameter increased from 141±5 to 169±6 after hemorrhage in the second series of animals.

Analysis, as appropriate. If the F value was significant, the Student-Neuman-Keuls test was performed. An α level of p<0.05 was considered significant in all statistical tests. Values are represented as mean±SEM of raw values or as percent changes from control values.

**Results**

Topically applied LVP (10–1,000 μU/ml) elicited concentration-dependent increases in pial arteriolar diameter. Responses obtained initially and 30 minutes later were comparable (Table 1). These responses were short in onset (30–45 seconds) and modest in duration (1–3 minutes). The changes in diameter represent 9±1%, 14±1%, and 18±2% increases in pial arteriolar diameter. Systemic arterial pressure was unchanged after topical LVP (73±4 vs. 72±4 mm Hg). In another group of animals, it was observed that the CSF vehicle for LVP had no effect on pial arteriolar diameter (125±6 vs. 124±5 μm) or systemic arterial pressure (66±4 vs. 66±4 mm Hg).

To determine the influence of a physiological intervention that increased pial arteriolar diameter...

![Figure 1. Influence of lysine vasopressin (LVP) (10–1,000 μU/ml) on pial arterioles during normotension (control) and hemorrhagic hypotension (blood withdrawn, 15 ml/kg). n=9. *p<0.05 compared with corresponding values before hemorrhage. Values are mean±SEM.](http://circres.ahajournals.org/doi/10.1161/01.RES.64.1.138)
Figure 2. Influence of LVP (100 μU/ml), PGE$_2$ (10 ng/ml), and coadministration of LVP (100 μU/ml), PGE$_2$ (10 ng/ml) upon pial arteriolar diameter in indomethacin-pretreated (5 mg/kg i.v.) piglets. n=6. *p<0.05 compared with corresponding control (c). In the case of PGE$_2$+LVP, PGE$_2$ alone serves as the control. Values are mean±SEM.

Upon responses to LVP, hemorrhagic hypotension was induced by withdrawing 15 ml blood/kg in another group of animals. Hemorrhage decreased systemic blood pressure from 68±3 to 29±1 mm Hg and increased pial arteriolar diameter from 154±7 to 181±8 μm, an 18±4% increase in diameter (Figure 1). Hemorrhage did not, however, alter PO$_2$, PCO$_2$, or pH. Normotension values were 32±2 mm Hg, 32±2 mm Hg, and 7.48±0.03, while hypotension values were 77±2 mm Hg, 31±1 mm Hg, and 7.49±0.03 for PO$_2$, and PCO$_2$, and pH, respectively (n=9). This pial arteriolar dilation was accompanied by an increase in CSF levels of 6-keto-PGF$_{1α}$, PGE$_2$, TXB$_2$, and PGF$_{2α}$. Normotension values were 934±108, 1,321±62, 405±14, and 1,223±38 pg/ml, while hypotension values were 2,889±180, 3,702±442, 771±40, and 3,185±474 pg/ml for 6-keto-PGF$_{1α}$, PGE$_2$, TXB$_2$, and PGF$_{2α}$, respectively. In contrast to normotensive conditions, under hemorrhagic hypotensive conditions, LVP elicited concentration-dependent decreases in diameter (Table 1, Figure 1). At the highest concentration studied (1,000 μU/ml), the pial arterioles constricted to the diameters observed before hemorrhagic hypotension (Figure 1).

Since hemorrhagic hypotension increased pial arteriolar diameter and enhanced CSF prostanoid synthesis, the ability of exogenously administered PGE$_2$ to alter the nature of the response to LVP was assessed in another series of experiments. These animals were pretreated with indomethacin (5 mg/kg i.v.) to prevent endogenous prostanoid production from altering arteriolar diameter and vascular responses. LVP (100 μU/ml, a midrange dose) and PGE$_2$ (10 ng/ml) produced pial arteriolar dilation when administered alone. In contrast, LVP (100 μU/ml) elicited arteriolar contraction when coadministered with PGE$_2$ (10 ng/ml) (Figures 2 and 3). The diameter resulting from the coadministration of LVP and PGE$_2$ was similar to the diameter in the control condition before administration of LVP (Figure 2). Topical PGE$_2$ had no effect on systemic arterial pressure (66±2 vs. 66±3 mm Hg).

To determine if a prostanoid-dependent mechanism of pial arteriolar dilation is required to reverse vascular responses to LVP, isoproterenol, a dilator that does not increase prostanoid synthesis, was used to increase pial arteriolar diameter. Topical LVP (100 μU/ml) and isoproterenol (30 ng/ml) elicited dilation when administered alone, whereas the LVP-induced dilation was reversed to contraction when LVP was coadministered with isoproterenol (Figure 4). Similar to hemorrhagic hypotension and topical PGE$_2$, coadministration of LVP...
and isoproterenol resulted in a diameter no different from the control condition before administration of LVP (Figure 4). Topical isoproterenol had no effect on systemic arterial pressure (69±4 vs. 67±3 mm Hg). After intravenous administration of indomethacin (5 mg/kg), LVP and isoproterenol responses were unchanged when administered alone, but LVP coadministered with PGE2 produced significantly greater arteriolar contraction as compared with responses in the absence of indomethacin (Figure 4). The CSF vehicle for isoproterenol had no effect on pial arteriolar diameter. All these mechanisms for increasing pial arteriolar diameter resulted in similar percent increases in arteriolar diameter (18±4, 24±2, and 24±2 for hemorrhagic hypotension, PGE2, and isoproterenol, respectively).

To determine if enhanced vasoconstriction of pial arterioles following dilation is a generalized phenomenon or specific for LVP, responses to norepinephrine and U46619 were determined prior to and following dilation with either hemorrhagic hypotension or PGE2. Norepinephrine and U46619 elicited concentration-dependent contractions of pial arterioles that were reproducible over time (Table 1). Topical norepinephrine and U46619 had no effect on systemic arterial pressure (71±2 vs. 70±3 mm Hg). Hemorrhage potentiated the constrictor responses to topical norepinephrine and U46619 (Table 2). The responses to norepinephrine were 6±0%, 12±1%, 16±2%, and those to U46619 were 15±1%, 26±1%, and 37±2% decreases in diameter before hemorrhage, whereas responses after hemorrhage were 13±1%, 21±1%, and 30±2% for norepinephrine, and 27±2%, 39±2%, and 59±2% for U46619. The concentration of PGE2 that reversed LVP-induced dilation to constriction also enhanced the contractile responses to norepinephrine and U46619 (Figure 3).

LVP-induced dilation was associated with increases in CSF levels of 6-keto-PGF1α, PGE2, TXB2, and PGF2α (Figure 5). However, LVP-induced dilation (Figure 1) was unchanged after indomethacin, 5 mg/kg i.v. (see below). With linear regression analysis, the slopes of the lines formed by application of LVP to animals not treated with indomethacin and to those treated with indomethacin were the same (control 0.015±0.003, indomethacin 0.017±0.004 μm/μL LVP). The intercepts, however, were different (control 137±5 μm, indomethacin 124±4 μm), consistent with the observation that indomethacin has been previously shown to elicit pial arteriolar constriction.15

Although LVP elicited increases in prostanoid levels under normotensive conditions, LVP did not produce a further increase in prostanoid levels under hemorrhagic hypotensive conditions. Indomethacin (5 mg/kg i.v.) potentiated LVP-induced constriction under hemorrhagic hypotensive conditions (Figure 6). The responses before indomethacin represent 9±1%, 15±1%, and 19±1% decreases in diameter, whereas responses after indomethacin represent 20±2%, 27±3%, and 33±3% decreases in diameter.

Dilation induced by LVP was blocked by intravenous administration of MEAVP, 5 μg/kg, a putative V1 (vascular) receptor antagonist (Figure 7). In addition to altering the vascular effects of LVP,
MEAVP also blocked the increased CSF prostanoid synthesis associated with topically applied LVP. The LVP-induced constriction under hemorrhagic hypotensive conditions was also blocked by MEAVP, 5 μg/kg i.v. (Figure 8). Pial arteriolar diameter and systemic arterial pressures of piglets treated with MEAVP were not different from before treatment.

To assess the selectivity of \( V_1 \) receptor blockade, responses to topical norepinephrine (30–300 ng/ml) and U46619 (3–30 ng/ml) were obtained before and after intravenous administration of MEAVP. Responses to norepinephrine and U46619 were unchanged after MEAVP (Table 3). The CSF vehicles for norepinephrine and U46619 had no effect on pial arteriolar diameter.

Discussion

Results of the present study show that LVP elicits pial arteriolar dilation under control conditions but constricts pial arterioles previously dilated by physiological and pharmacological intervention. LVP-induced dilation is accompanied by increased cortical periarachnoid CSF levels of 6-keto-PGF\(_{1\alpha}\), PGF\(_2\), TXB\(_2\), and PGF\(_{2\alpha}\), but LVP-induced pial arteriolar dilation was unchanged after indomethacin. Therefore, increased dilator prostanooid synthesis cannot account for LVP-induced cerebrovasodilation. However, LVP-induced constriction of previously dilated arterioles is potentiated by indomethacin (5 mg/kg). This dose of indomethacin reduces cortical periarachnoid fluid prostanoid concentration and inhibits the conversion of exogenous arachidonic acid to prostanoids by more than 90%.\(^{15,17}\) These data suggest that the response to LVP in the cerebral circulation is tone-dependent and that prostanoids modulate constrictor responses but are not involved in dilator responses. Since pial arteriolar dilation through physiological and pharmacological manipulation enhanced constrictor responses to norepinephrine and U46619, a purported TX\(_A2\) mimic,\(^{18}\) other vasoactive mechanisms may be tone-dependent as well.

LVP-induced vascular and biochemical changes were blocked by MEAVP, a putative \( V_1 \) receptor antagonist.\(^{19,20}\) Since systemically administered MEAVP blocked the actions of LVP applied to the brain side of the blood-brain barrier, these data suggest that MEAVP can cross the blood-brain barrier in newborn pigs. MEAVP appears to produce selective blockade of \( V_1 \) receptors in the cerebral circulation since responses to norepinephrine and U46619 were unchanged after MEAVP.

Vasopressin is known to produce potent vasoconstriction in a variety of vascular regions,\(^{1–3}\) but the ability of vasopressin to influence cerebral hemodynamics is less well understood. Immunocytochemical studies have provided evidence for vasopressin as a
putative neurotransmitter or neuromodulator within the brain.4,5 Endogenous vasopressin may gain access to brain blood vessels via two different mechanisms: the blood and the CSF. Although the contribution of plasma vasopressin is thought to be minimal in the adult,21 a selectively permeable blood-brain barrier is present at birth, and vasopressin may be able to cross the barrier in limited quantities.22,23 The origin of the CSF vasopressin is thought to be the hypothalamic vasopressin-containing neurons, and the CSF may serve as a transport system whereby vasopressin can reach various parts of the brain.24,25 The CSF vasopressin concentration under normotensive conditions has been reported to be approximately 4 μU/ml in dogs,21 cats,26 rats,27 and humans,28 while during hemorrhage, it may increase to as much as 242 μU/ml, as reported in the dog.29 We are presently unaware of published CSF values for pigs.

To date, reports on the cerebrovascular actions of vasopressin have been confusing and contradictory. Vasopressin has been variously reported to produce cerebral artery contraction in the cat, goat, human, and rat,30-33 dilation in the cat and dog,34,35 or no effect in the rat.36 Additionally, infusion of vasopressin into the internal carotid artery of rats is followed by an increase in cerebral blood flow,37 whereas decreases in cerebral blood flow are observed when vasopressin is infused into the internal maxillary artery of the goat.31 The dilation elicited by vasopressin in the canine basilar artery has been reported to be endothelium-dependent.35,38 MEAVP is capable of blocking both this dilation and vasopressin-induced femoral artery contractions.35,38 Atypical responses to vasopressin have been observed in other vascular beds, where vasopressin elicits human forearm vasodilation and reverses hypoxic pulmonary hypertension in the rat,39-40 Moreover, vasopressin elicits pulmonary and peripheral vasodilation when administered after V1 receptor blockade in conscious rats and dogs.41,42 The latter vasodilation was unchanged after cyclooxygenase blockade, and its mechanism is undetermined.41,42

Prostanoids are prominent in the cerebrovascular physiology of the neonate and appear to contribute to the maintenance of resting cerebral blood flow.8 Previously, we found that there is an important interaction between neurotransmitters such as acetylcholine, norepinephrine, and histamine such that these neurotransmitters increase cerebral prostanoid production and that the production influences cerebral vascular responses.9-11 In addition, brain prostanoids increase vasopressin secretion in the conscious rat,43 and prostanoids are thought to be involved in the vascular actions of vasopressin.12,13

Prostanoids can be involved in the cerebral hemodynamic response to vasopressin in the newborn...
pig. There is a temporally related increase in prostanoid synthesis associated with vasopressin-induced dilation. However, the failure of indomethacin to alter vasopressin-induced dilation indicates that an increase in prostanoid synthesis cannot account for this response. In contrast, prostanoids appear to modulate the vasopressin-induced constriction. Activation of V1 receptors is accompanied by an increase in the intracellular calcium concentration, which may mediate both prostanoid production and vascular smooth muscle contraction.13

Although the level of tone in the pulmonary circulation has been previously shown to alter the nature of the response to adrenergic and cholinergic stimuli, the mechanism for the alteration is uncertain.44-45 It is possible that changes in the physical state of cell membranes, which occur during vasoconstriction and dilation,46 may play a role in the tone-dependent nature of vascular responses. To our knowledge, no one has previously documented that responses are tone dependent in the cerebral circulation. The transition of vasopressin from a dilator to a constrictor in the cerebral circulation of the newborn pig may also depend on the relative concentrations of endothelium-dependent relaxant and contractile factors.35,38

Since the LVP concentrations chosen in this study are similar to concentrations that have been detected in CSF of several species, vasopressin could have a physiological role in the control of the cerebral circulation. The present study is the first to document that responses to vasopressin are tone-dependent in the cerebral circulation.

Acknowledgments

The authors thank Lori Doty, Joel Giddens, Deanne Hardy, Don Beasley, and Mildred Jackson for excellent technical assistance in performing the experiments. The authors also wish to thank Kris Arheart for excellent statistical analysis.

References


Table 3. Influence of MEAVP on Pial Arteriolar Responses to Norepinephrine and U46619 in the Newborn Pig

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<th>Agent</th>
<th>Control</th>
<th>MEAVP*</th>
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<td>Norepinephrine (ng/ml)</td>
<td>Arteriolar diameter (µm)</td>
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<td>148±6</td>
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n=5.

*[(1-β-mercaptop-β-cyclopentamethylene propionic acid,2- (O-methyl)Tyr-Arg (MEAVP)] 5 µg/kg i.v.

†p<0.05 compared with corresponding zero concentration value (0).
23. Mens WBJ, Witter A, Greidanus TBW: Penetration of neurohypophyseal hormones from plasma into cerebrospinal fluid (CSF): Half-times of disappearance of these neuropeptides from CSF. *Brain Res* 1983;262:143–149


**KEY WORDS** • cerebral circulation • pial arteriole • vasopressin • prostanoids • newborn
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