Age dependency of vasopressin pulmonary vasodilatory effect in rats

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Abstract

Background—Vasopressin is a systemic vasoconstrictor. Its pulmonary vasodilatory effect is controversial and limited data are available on its use in neonates with pulmonary hypertension. Hypothesizing that the vasopressin-induced pulmonary vasodilation is developmentally regulated, we evaluated its pulmonary and systemic arterial response in newborn and adult rats.

Methods—Vessels were mounted on a wire myograph and the vasopressin-induced changes in vasomotor tone measured. The vessel- and age-dependent differences in vasopressin V_{1a} and V_2 receptors expression were evaluated by Western blotting.

Results—Vasopressin induced a dose-dependent increase in mesenteric arterial tone at both ages, but of greater magnitude in adult vessels (P<0.01). At lower concentrations, vasopressin induced pulmonary vasodilation in adult vessels and vasoconstriction in newborn arteries. The adult vasopressin-induced pulmonary vasodilation was inhibited by ibuprofen suggesting that the response is prostaglandin mediated. Pulmonary tissue V_{1a} receptor protein expression was higher in adult, when compared with newborn arteries (P<0.01). The adult vessels V_{1a} expression predominated in the pulmonary arteries and V_2 was only detected in mesenteric arteries.

Conclusion—The vasopressin-induced pulmonary vasodilation is absent in newborn rats likely due to the lower tissue V_{1a} expression early in life. These animal data challenge the therapeutic use of vasopressin in neonatal pulmonary hypertension.

Introduction

Vasopressin, also known as 8-arginine-vasopressin or antidiuretic hormone, is of a neurohypophysial origin and has an important neurotransmitter role on the control of circadian rhythm, thermoregulation and adrenocorticotrophic hormone (ACTH) release (1). Aside from these central effects, vasopressin acts in a circulation-specific manner to modulate vascular tone. It induces systemic vasoconstriction and this effect led to its therapeutic use in clinical conditions where reduced systemic vascular resistance is a concern, such as septic shock (1, 2). Yet, vasopressin has also been shown to reduce

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pulmonary vascular resistance in human and animals (3–6) leading to its use in the treatment of pulmonary arterial hypertension in adults (3, 4). A recent review of the available clinical literature did not find sufficient evidence to recommend vasopressin as a pulmonary vasodilator in adults (7).

Persistent pulmonary hypertension syndrome of the newborn (PPHN) is a clinical condition associated with high morbidity and mortality (8). Inhaled nitric oxide remains the preferred therapeutic strategy to treat infants with the PPHN syndrome given its preferential vasodilatory effect on the pulmonary vasculature (8). Yet, a number of pharmacological agents are also commonly utilized in the treatment of this disorder (8). In spite of limited data to justify its clinical use as a pulmonary vasodilator, vasopressin has been employed in the treatment of infants with PPHN syndrome (9).

Aside from the paucity of data in support of the therapeutic use of vasopressin in the infants with pulmonary hypertension, there is reason to dispute the claim of this drug having a pulmonary vasodilatory effect early in life. In rats, the tissue vasopressin receptors' mRNA expression is developmentally regulated and the V_{1a} subunit potentially involved in its vasodilatory response is not present in newborn lungs (10).

Therefore, the purpose of the present study was to conduct an *in vitro* comparative evaluation of the effect of vasopressin on the pulmonary and systemic vascular tone of newborn and adult rats. We hypothesized that vasopressin does not have a pulmonary vasodilatory effect in the newborn.

Results

Vasopressin effect on mesenteric and pulmonary arterial vasomotor tone

We first evaluated the vasopressin dose-response in adult mesenteric and pulmonary arteries without any agonist-induced prestimulation. Vasopressin induced contraction of mesenteric arterial smooth muscle, but had no effect on the pulmonary arteries (Figure 1).

In order to further evaluate the potential relaxant effect of vasopressin all subsequent dose-response measurements were obtained in vessels precontracted with the previously determined EC_{75} U46619 concentration. In mesenteric arteries, vasopressin induced a dose-dependent increase in smooth muscle contraction that was maximal for both ages at 6×10^{-9} M (Figure 2A). In contrast, the vasopressin effect on the pulmonary arterial vasomotor tone was age-dependent (Figure 2B). Whereas an incremental dose-dependent relaxation was observed in the adult arteries, vasopressin induced a dual response in the newborn pulmonary vessels. In the latter, vasopressin stimulation led to pulmonary arterial muscle contraction that was maximal at 3×10^{-9} M and vasorelaxation was only observed at the highest tested concentration (6×10^{-7} M).

Since tachyphylaxis has been reported in vasopressin-stimulated vessels *in vitro*, we evaluated the mesenteric and pulmonary arterial response to a single high concentration of the drug (6×10^{-7} M). The vasopressin response to the single dose was similar to the one observed following multiple incremental concentrations. In mesenteric arteries only muscle

contraction was observed at both ages, whereas in the pulmonary tissue, vasorelaxation was seen in adult vessels and the opposite in the newborn arteries (Figure 3).

Mechanism accounting for the vasopressin-induced pulmonary vasorelaxation

We further proceeded to investigate the mechanism involved in the vasopressin-induced relaxation of adult pulmonary arteries. Two main pathways have been suggested as being involved in the regulation of vasopressin-induced vasodilation: endothelium-dependent nitric oxide synthase-derived nitric oxide and vasodilating prostaglandins (12, 13). As such, we evaluated the impact of inhibitors of both pathways on the vasopressin-induced pulmonary vasodilatory response. The cyclooxygenase inhibitor ibuprofen, but not the nitric oxide synthase blocker L-NAME significantly (P<0.01) inhibited the vasopressin-induced pulmonary vasorelaxation (Figure 4). In the presence of ibuprofen, vasopressin induced pulmonary vasorelaxation at concentrations greater than 10^{-7} M, yet of a lesser magnitude when compared with control untreated vessels.

A comparative evaluation of the pulmonary and mesenteric artery vasopressin V_{1a} and V_2 receptors expression was conducted. As shown in Figure 5, pulmonary and mesenteric expression of the V_{1a} receptor is tissue and age dependent. Whereas a higher V_{1a} receptor expression was documented in adult, when compared with newborn pulmonary arterial tissue, the opposite age-dependent pattern was observed in mesenteric vessels (Figure 5 A and B). When the endothelial and smooth muscle cell-specific expression of the V_{1a} receptor was evaluated (Figure 5 C–F), no age-dependent changes were observed in muscle cells, whereas the endothelial receptor expression pattern mirrored the whole tissue data. We further comparatively quantified the adult pulmonary and mesenteric arterial tissue V_{1a} expression and observed that the former has a higher (P=0.02) V_{1a} receptor expression, when compared with mesenteric vessels (V_{1a}/GAPDH of 1.9 ± 0.4; N=4 and 0.5 ± 0.2 arbitrary units; N=4 respectively).

Lastly, we quantified the vasopressin V_2 receptor protein expression in newborn and adult pulmonary and mesenteric arterial rat tissue (Figure 6). In pulmonary vessels, V_2 receptor expression was undetectable with the commercially available antibody used (Panel A), whereas its expression in mesenteric vessels was significantly higher in adult, when compared with newborn tissue (Panel B; P<0.05).

Discussion

Vasopressin is a powerful systemic vasoconstrictor commonly utilized clinically to reverse shock unresponsive to other vasopressors (2). On the basis of its potential pulmonary vasodilator effect, this drug has been increasingly employed in the treatment of pulmonary hypertension in adults (1) and infants with the PPHN syndrome (9).

In the present study, we evaluated the age and circulation-specific effects of vasopressin on the newborn and adult rat vascular tone. In adult rats, *in vitro*-exposure to vasopressin led to a significant increase in mesenteric arterial tone and dose-dependent pulmonary arterial vasorelaxation. In contrast, vasopressin stimulation of newborn rat vascular tissue, resulted in increased pulmonary vasomotor tone at low doses and pulmonary vasorelaxation only at

the maximal tested concentration (6×10^{-7} M). The observed vasopressin-induced increase in mesenteric vasomotor tone, although of lesser magnitude in newborn arteries was present at both tested ages.

The pulmonary vasodilatory effect of vasopressin in adult animals is species-dependent. It is present in dogs (14), absent in rabbits (15) and in guinea pigs only documented in pulmonary veins (16). To some extent this species-related differences in vasopressin response are secondary to the experimental conditions under which the vessels were tested. In published reports where vasopressin failed to induce pulmonary arterial vasodilation the drug effect was evaluated in vessels lacking basal vasomotor tone (15, 16). Vasopressin has also been reported to vasorelax systemic vessels such as the human forearm vasculature (17) and cerebral vessels of distinct adult animal species (18).

Precontracted pulmonary arteries of adult rodents exhibit vasodilation in response to vasopressin (19, 20), whereas vasoconstriction in response to a very high drug concentration (10^{-5} M) was reported in unstimulated mouse pulmonary vessels (21). In the present study, we did not observe any vasopressin-induced vasomotor effect in adult rat pulmonary arteries lacking basal agonist-stimulated tone, even when exposed to concentrations as high as 6×10^{-7} M.

Vasopressin acts on three receptors: V_{1a}, V_{1b} and V_2 (22). The V_{1a} receptor is present in vascular tissue endothelial and smooth muscle cells and considered responsible for the vasopressin-induced vasoconstriction and vasodilation (14, 22). V_2 is the main vasopressin receptor isotype in renal parenchymal tissue (23), while its expression in rat lung is controversial (24). The V_{1b} (or V_3) is expressed in the anterior pituitary gland and involved in ACTH secretion (25).

In the present study we confirmed that in rats, V_{1a} receptor expression is present in both pulmonary and mesenteric arterial tissue endothelial and smooth muscle cells, but exhibiting a circulation- and age-specific distinct pattern. Comparing adult rat vascular tissue, we showed that V1a expression is higher in pulmonary, as opposed to mesenteric arteries.

We were unable to detect significant V_2 receptor expression in pulmonary arterial tissue at both tested ages. In the present study, mesenteric arterial V_2 receptor expression was found to be age-dependent and highest in adult, as compared with newborn tissue. Together these data suggest that V_{1a} is responsible for pulmonary arterial vasorelaxation, whereas the V_2 receptor modulates the vasopressin constrictor response that is predominant in mesenteric vessels, when compared with pulmonary arteries.

The present data strongly suggest that the age-related dependency of the vasopressin-induced pulmonary vasorelaxation is caused by the significantly lower expression of vasopressin V_{1a} receptor in newborn, as compared with adult lung vascular endothelial cells. A previous report showing evidence for V_{1a} receptor mRNA expression in adult, but not newborn rat lungs (10) further supports this conclusion. In keeping with the present evidence, vasopressin administration (20mIU/kg/min) to newborn goats induced a significant increase in pulmonary vascular resistance (26). Together these data strongly suggest that in newborn animals, vasopressin has no pulmonary vasodilatory effect.

The mechanism by which vasopressin induces pulmonary vasodilation in adult animals is controversial. The presence of an intact endothelium is required for vasopressin to induce pulmonary vasorelaxation (14). Endothelial-derived nitric oxide (6, 27) has been incriminated as the mediator of vasopressin-induced pulmonary vasodilation. Yet vasopressin stimulation did not alter nitric oxide generation in rat aorta (28). In rat (29) and canine (30) systemic arteries, cyclooxygenase inhibition potentiates the vasopressin-induced vasoconstriction suggesting that prostaglandins are involved in the vasopressin regulation of vasomotor tone. Sai *et al* observed that while the adult canine pulmonary arterial vasodilation to vasopressin was nitric oxide-dependent, prostacyclin was involved in the drug-induced pulmonary vein relaxation (5).

In the present study, the cyclooxygenase inhibitor ibuprofen, but not the nitric oxide synthase blocker L-NAME suppressed the vasopressin-induced pulmonary vasorelaxation at lower drug concentrations. In fact, in the presence of ibuprofen, vasopressin enhanced pulmonary vasomotor tone suggesting that it unmasked a direct effect of this drug on the vessels' smooth muscle contractile potential. Lastly, there is evidence that prostaglandins regulate the vasomotor tone of certain vascular beds through the V_2 receptor. Medina *et al* (31) documented that the V_2 receptor agonist desmopressin caused endothelium-dependent relaxation in human renal arteries and this vasodilatory effect was inhibited by indomethacin. Since in the present study we were unable to document V_2 receptor expression in lung vascular tissue it is unlikely that the vasopressin-induced pulmonary vasodilation in rats is modulated via this receptor.

Clinical studies in adult subjects have not consistently shown an effect of vasopressin on the pulmonary vasculature (3). Similarly, little is known about the vasopressin effect on the newborn pulmonary vasomotor tone with its pulmonary vasodilatory therapeutic effect in infants mostly appearing as clinical case-reports. Scheurer *et al* (4) described two neonates that showed elevated pulmonary arterial pressure following surgical correction of their anomalous pulmonary veins. Both infants were treated with nitric oxide and one of them with the phosphodiesterase 3 inhibitor milrinone prior to the addition of vasopressin, making it difficult to confirm the vasopressin pulmonary vasodilatory effect (4). Stathopoulos *et al.* (32) recently reported on the effect of a long acting analogue of vasopressin (terlipressin). The drug was shown to improve the patient's systemic arterial pressure and reduce the echocardiographic estimate of elevated pulmonary arterial pressure in a newborn with congenital diaphragmatic hernia (32). Given such limited evidence for its potential pulmonary vasodilatory effect, it is rather surprising that a significant number of neonatologists acknowledge its therapeutic use in the PPHN syndrome (9).

Lastly, the extent to which the *in vitro* vasopressin concentrations utilized in the present study reflect *in vivo* therapeutic serum levels in humans deserves further comments. The physiological serum vasopressin levels in adult human subjects is 2.22 pg/ml (33). In adult dogs, a basal plasma vasopressin level of 2.3 ± 0.4 pg/ml was reported that increased to 280 ± 23 pg/ml following vasopressin infusion (7.6 ng/kg/min)(34). In newborn sheep the physiological vasopressin levels have been reported to be in the range of 7.0 pg/ml (35). In the present study, we evaluated *in vitro* vasopressin concentrations ranging from 1.1– 660,000 pg/ml (10^{-12}–6×10^{-7} M). The adult pulmonary arteries only exhibited significant

vasorelaxation at vasopressin concentrations 3,300 pg/ml. Thus, to the extent that the adult rat pulmonary arteries evaluated *in vitro* reflect the *in vivo* conditions, vasopressin concentrations higher than commonly utilized clinically are required to induce significant pulmonary vasodilation.

In summary, whereas vasopressin has a significant systemic vasoconstrictor effect in newborn and adult rats, the pulmonary vasodilatory response of this drug is age-dependent. The reduced expression of V_{1a} receptors in the pulmonary arterial tissue of the newborn, as compared with adult rat, likely accounts for the lack of vasopressin-induced pulmonary vasodilation early in life. These animal data, together with the limited clinical evidence in support of vasopressin having a significant pulmonary vasodilatory effect early in life, raise concerns about its therapeutic use in infants with the PPHN syndrome. Further studies attempting to validate the present animal data in newborn and adult human tissue are warranted.

Materials and Methods

Animals

All procedures were conducted according to criteria established by the Canadian Council on Animal Care and were approved by the Animal Care Committees of The Hospital for Sick Children Research Institutes.

Newborn (2–7 days of age) and adult (> 60 days old) Sprague-Dawley rats were studied. The animals were sacrificed with an overdose of pentobarbital sodium (50 mg/kg i.p., BHD Inc., Toronto, ON, Canada) and the lungs, as well as mesenteric bed was quickly removed and maintained on an ice-bed for further dissections.

Near-resistance (3rd–4th generations) intrapulmonary and mesenteric arteries were isolated and mounted on a wire myograph (Danish Myo Technology A/S, Aarhus, Denmark). The vessels were bathed in Krebs-Henseleit buffer (NaCl, 115 mM; NaHCO3, 25 mM; NaHPO4, 1.38 mM; KCl, 2.51 mM; MgSO4 - 7 H2O, 2.46 mM; CaCl2, 1.91 mM; and dextrose, 5.56 mM) bubbled with air/6% CO2 and maintained at 37°C.

Organ bath study

The functional evaluation of pulmonary and mesenteric arteries has been previously described (11). Briefly, lung intralobar pulmonary or mesenteric artery ring segments (average diameter 80–100 μm and length=2 mm) were dissected free and mounted on a wire myograph. Isometric changes were digitized and recorded online (Myodaq, Danish Myo Technology A/S,). After 1 h of equilibration, the optimal vessel resting tension was determined by repeated stimulation with 128 mM KCl until maximum active tension was reached. All subsequent force measurements were obtained at optimal resting tension.

The vascular muscle precontraction was induced with the pre-determined effective concentration to induce 75% of maximal contraction (EC_{75}) with the thromboxane A_2-mimetic U46619 (Cayman Chemical Inc, Ann Arbor, MI). The newborn and adult U46619 EC_{75} concentrations were 4×10^{-7} M and 2×10^{-7} M for the pulmonary arteries and 6×10^{-7}

M and 4×10^{-8} M for the mesenteric vessels, respectively. The vasopressin (Pharmaceutical Partners of Canada Inc., Richmond Hill, Ontario, Canada)-induced changes newborn and adult pulmonary and mesenteric vascular tone were determined.

Western blotting

Primary endothelial and smooth muscle cells retrieved from intrapulmonary and mesenteric arteries, as well as the whole tissue extracts were utilized to measured vasopressin V_{1a} and V_2 receptors expression.

For vascular endothelial cells isolation the tissue digested with 1 mg/ml of collagenase type II (Sigma-Aldrich, Oakville, Ontario, Canada) for 2h at 37°C. The digest was then passed through 70 μm cell strainer to remove tissue fragments, pelleted by centrifugation at 200 G for 10 min and resuspended with 2% fetal bovine serum (FBS, Gibco) in phosphate buffered solution containing 5μl biotinylated rat anti-mouse CD31 antibody (BD, PharMingen, San Diego, CA). After incubation on ice for 1h, the endothelial cells were immobilized with streptavidin magnetic beads (New England Biolabs, Ipswich, MA) on ice. The endothelial cells were then placed on the EasySep magnet (Stemcell technologies, Vancouver, BC, Canada) for 5 min and unbound cells were removed. Bound endothelial cells were lysed in 10 mmol/l Tris–HCl pH 7.4 lysis buffer-containing 1% Triton X-100 and protease/ phosphatase inhibitors (Roche Diagnostics Canada, Laval, Quebec, Canada) and centrifuged at 13000g for 30 min.

For smooth muscle isolation, the arteries were digested with 1mg/ml collagenase type II for 2h, pelleted at 200G for 10 min, and washed with growth medium composed of DMEM (Wisent, Montreal, Quebec, Canada) supplemented with 10% FBS (Wisent, Montreal, Quebec, Canada) and 2.5% penicillin/streptomycin/fungizone. The pellet was resuspended in growth medium and incubated at 37C, 5%CO2 with 90% humidity followed by media changes at 24 hours and every 4 days until confluence. Cells were utilized at passage 2.

For the whole tissue protein extraction, the pulmonary and mesenteric arteries were lysed in 10 mmol/l Tris–HCl pH 7.4 lysis buffer-containing 1% Triton X-100 and protease/ phosphatase inhibitors (Roche Diagnostics Canada, Laval, Quebec, Canada) and centrifuged at 13000g for 30 min. Equal amounts of lysate proteins in Laemmli buffer were separated by SDS-PAGE and immunoblotted, as previously described using the following antibodies: rabbit Vasopressin-V_{1a} (AVPV1a) and V_2 (AVPV2A) (Alpha Diagnostic, San Antonio, Texas), mouse GAPDH (Sigma-Aldrich, Oakville, Ontario, Canada), anti-mouse IgG peroxidase conjugated (Sigma-Aldrich, Oakville, Ontario, Canada) and anti-rabbit IgG HRP-conjugated (Cell Signaling Technology, Danvers, MA). Detection was performed with the enhanced chemiluminescence reagent (Perkin Elmer, Shelton, CT). Band intensities were quantified using ImageJ (National Institutes of Health-NIH, Bethesda, Maryland) and expressed relative to GAPDH.

Statistical Analysis

Data were evaluated by one or two-way analysis of variance (ANOVA) with multiple comparisons obtained by the Tukey-Kramer test. One comparing only 2 groups, Student t-test was employed. Statistical significance was accepted at $P < 0.05$. All statistical analyses

were performed with the Number Cruncher Statistical System (NCSS, Kaysville, Utah). Data are presented as means ± SEM.

Acknowledgments

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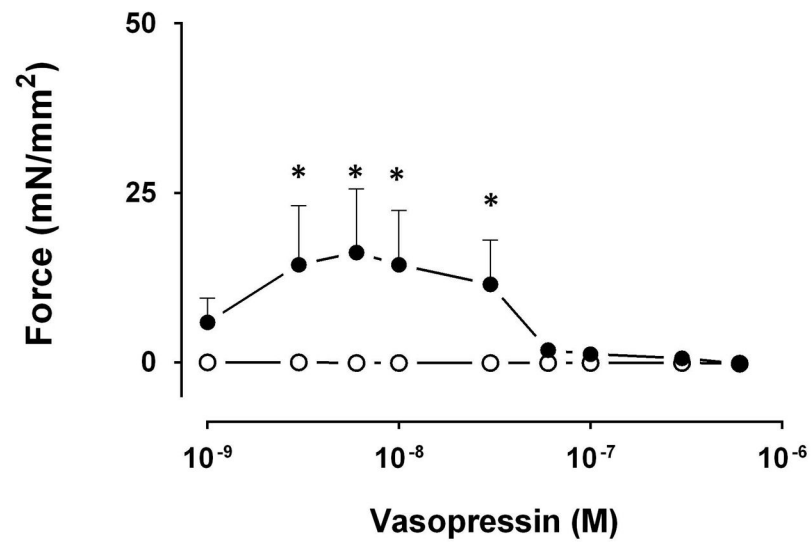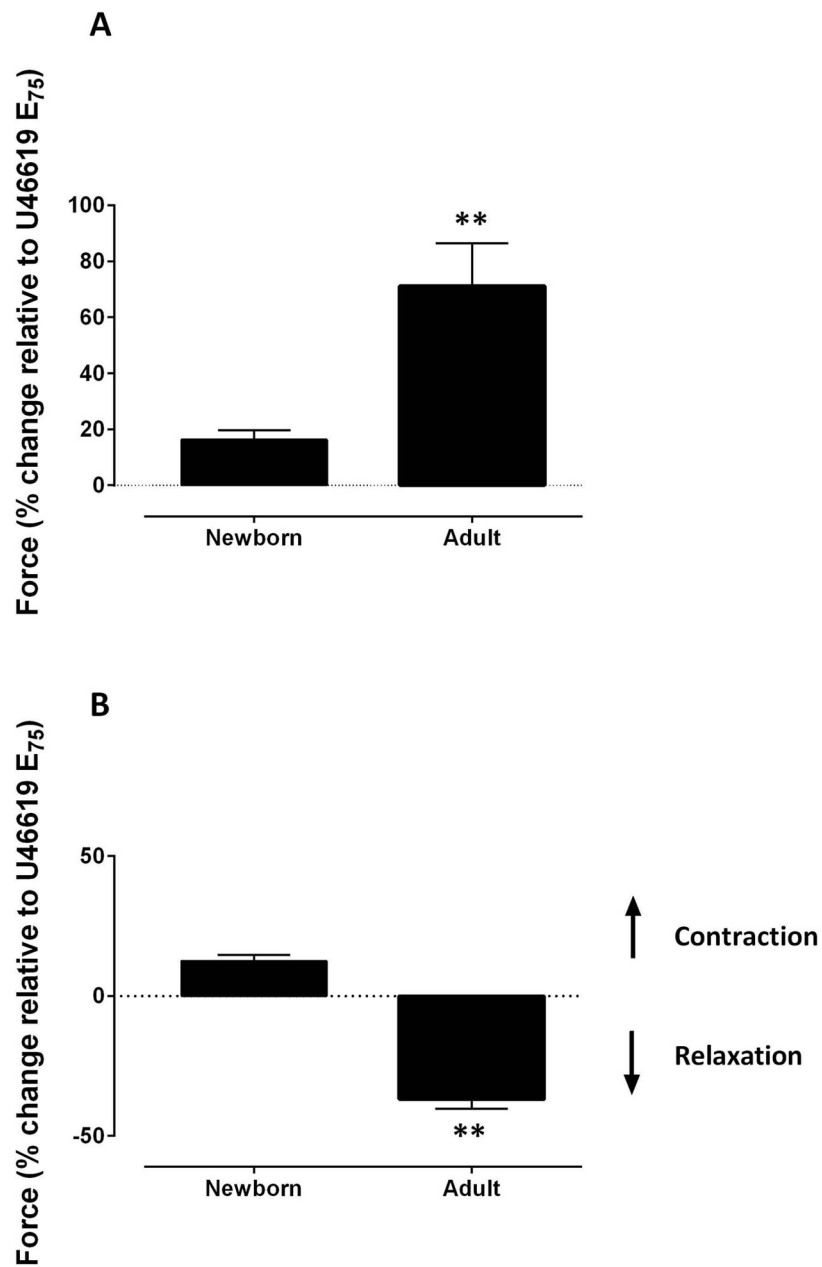
Figure 1.
Vasopressin effect in adult pulmonary and mesenteric arteries without agonist-induced tone. Non precontracted adult pulmonary (N=4) and mesenteric arteries (N=4) response to vasopressin. Open circles = Pulmonary Artery; Solid Circles = Mesenteric Artery. *P<0.05 by One-way ANOVA with repeated measures and Fisher's least-square-difference multiple-comparison test.
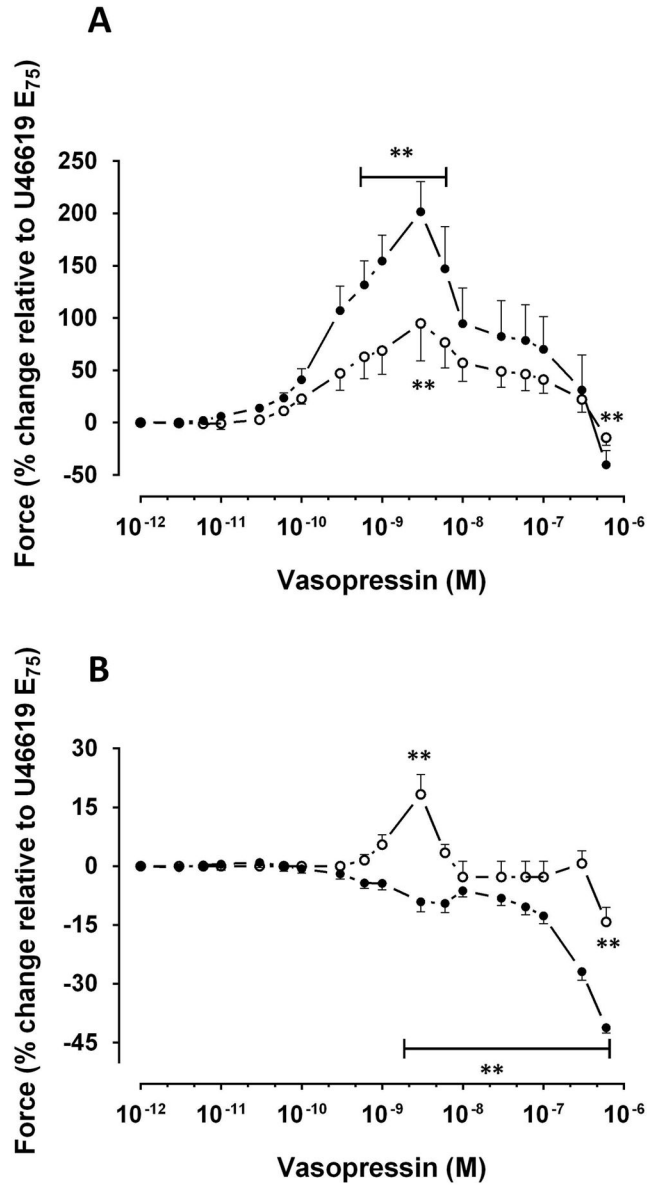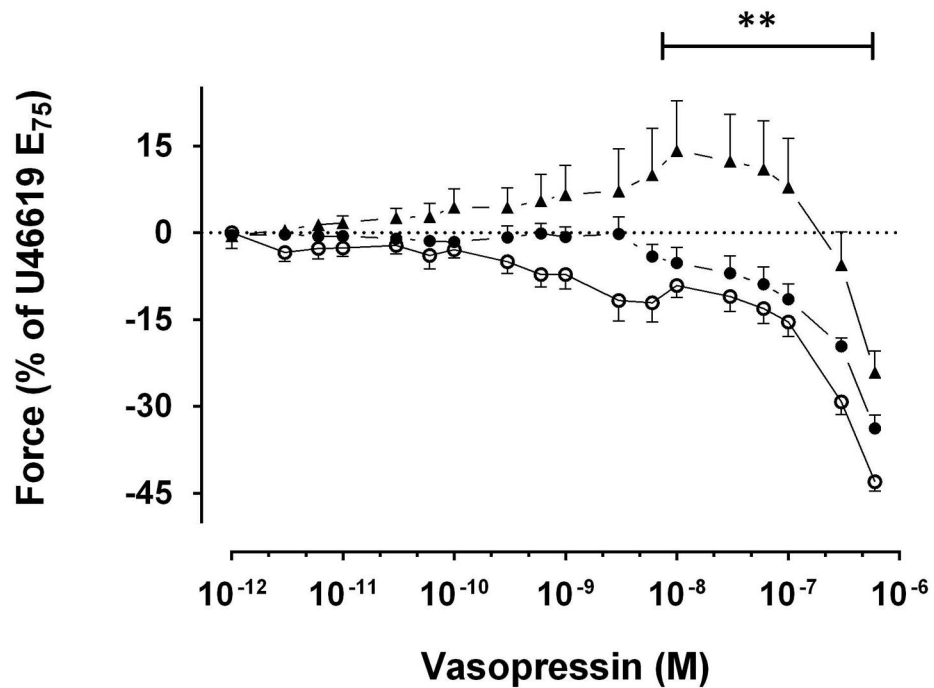
Figure 2.
Vasopressin induced vasomotor tone changes in newborn and adult pulmonary and mesenteric arteries. Vasopressin dose-response in thromboxane A_2 analogue (U46619) precontracted (EC_{75} concentration) newborn and adult mesenteric (Panel A. N=4 each) and pulmonary arteries (Panel B. Newborn N=6; Adult N=8). The vasopressin-induced changes are expressed as percentage of precontraction force values. Positive values indicate vasopressin-induced contraction and negative values indicate vasorelaxation. Open circles = Newborn; Solid circles = Adult. ** P< 0.01 versus respective initial 10^{-12} M values by two-way ANOVA and Tukey-Kramer multiple comparison testing.

A

B

Figure 3.
Vasopressin single dose effect on newborn and adult pulmonary and mesenteric arterial tone. Single concentration (6×10^{-7} M) vasopressin-induced force in Newborn and adult mesenteric (Panel A. Newborn N=3; Adult N=4) and pulmonary arteries (Panel B. Newborn N=4; Adult N=4) precontracted with thromboxane A_2 analogue (U46619 (EC_{75} concentration). Vasopressin-induced response is expressed as % change from U46619-induced force and negative values indicate relaxation. ** P< 0.01 versus newborn values by unpaired Student t-test.
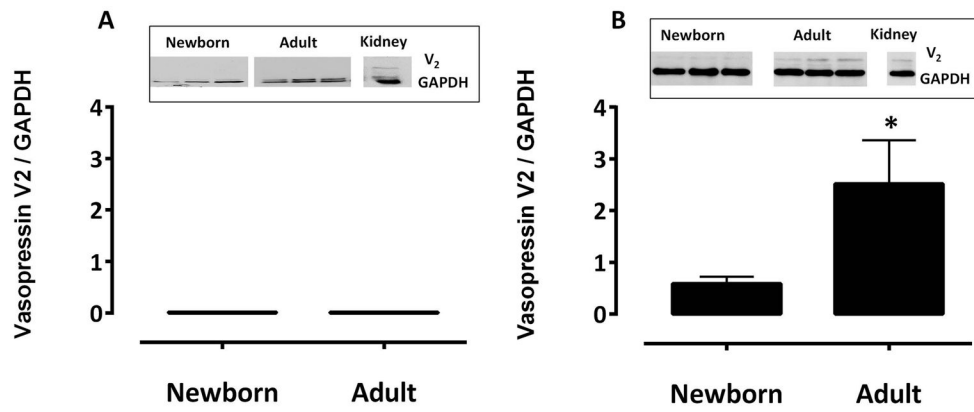
Figure 4.
Vasopressin pulmonary vasodilatory effect and cyclooxygenase/NOS inhibition. Vasopressin dose-response in Thromboxane A_2 analogue (U46619) precontracted (EC_{75} concentration) adult pulmonary arteries in the absence (Control; N=8) and presence of either the cyclooxygenase inhibitor ibuprofen (10^{-4} M; N=4) or the nitric oxide synthase blocker L-NAME (10^{-4} M; N=4). Positive values indicate vasopressin-induced contraction and negative values indicate vasorelaxation. Open circles = Control; Solid circles = L-NAME; Solid triangles = Ibuprofen. ** P< 0.01 relates to ibuprofen data significantly different when compared with control vessels at the same vasopressin concentrations by two-way ANOVA and Tukey-Kramer multiple comparison testing.
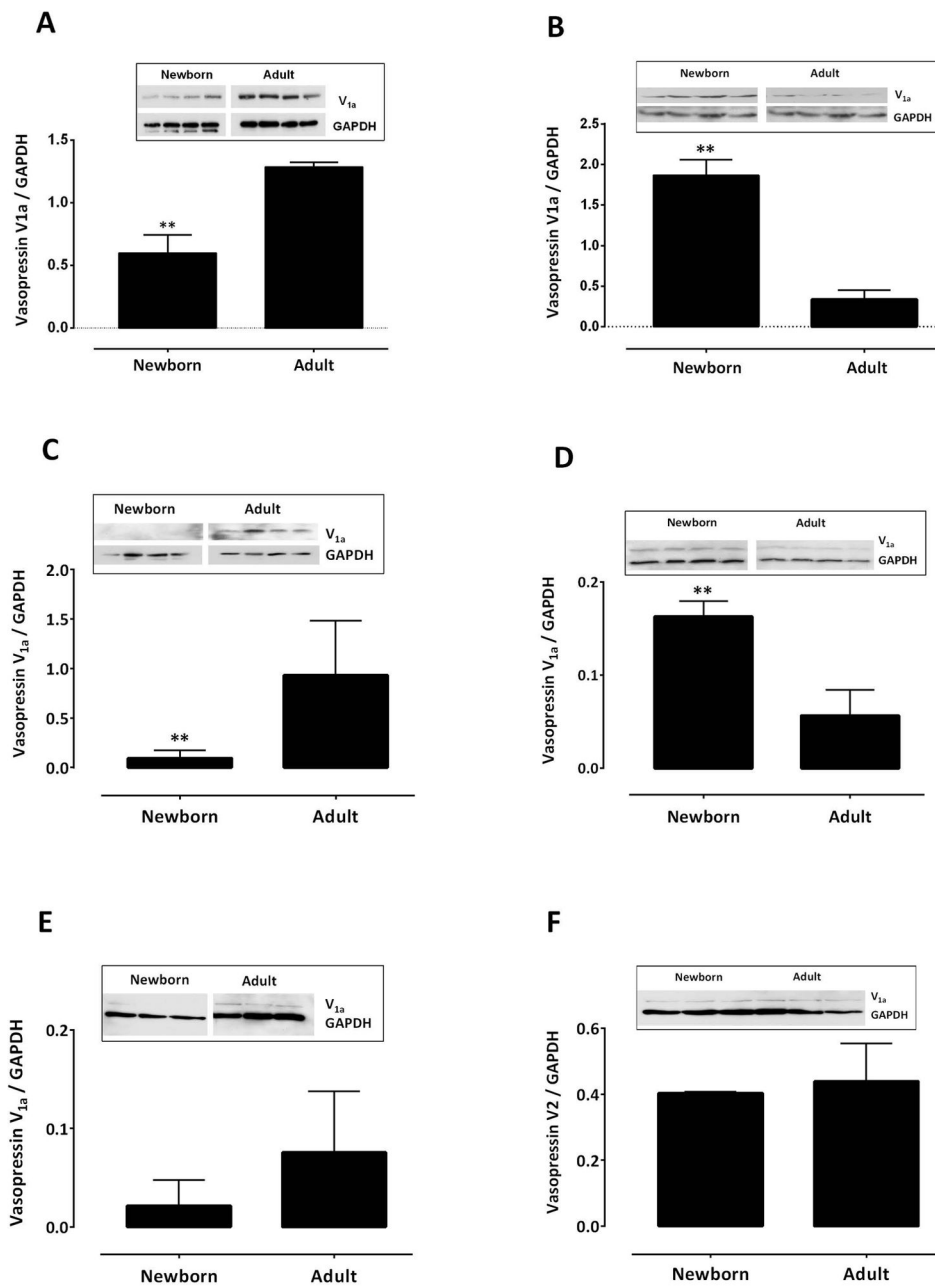
Figure 5.
Pulmonary and mesenteric tissue, endothelial and smooth muscle cell vasopressin V_{1a} receptor protein expression. Newborn and adult rat 3rd-4th generation pulmonary (Panel A; N=4 each) and mesenteric arterial tissue (Panel B; N=4 each), as well as their respective primary endothelial (Panels C and D; N=4 each) and smooth muscle cells (Panels E and F; N=3 each). Vasopressin V_{1a} receptor was determined by Western blotting and normalized to the tissue and cell GAPDH content. ** $P< 0.01$ versus adult values by unpaired Student t-test. Representative Western blots are illustrated.

Figure 6.
Pulmonary and mesenteric arteries vasopressin V_2 receptor protein expression. Newborn and adult rat 3rd–4th generation pulmonary (Panel A; N=3 each) and mesenteric arterial tissue (Panel B; N=3 each). Vasopressin V_2 receptor was determined by Western blotting and normalized to the tissue and cell GAPDH content. Adult rat kidney tissue was used as a positive control * $P<0.05$ versus newborn values by unpaired Student t-test. Representative Western blots are illustrated.