Research report

Vasopressin impairs $K_{ATP}$ and $K_{ca}$ channel function after brain injury

Alana Salvucci, William M. Armstead*

Departments of Anesthesia and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA

Accepted 3 October 2000

Abstract

This study was designed to characterize the role of vasopressin in impaired pial artery dilation to activators of the ATP sensitive $K$ ($K_{ATP}$) and calcium sensitive $K$ ($K_{ca}$) channel following fluid percussion brain injury (FPI) in newborn pigs equipped with a closed cranial window. Topical vasopressin was coadministered with the $K_{ATP}$ and $K_{ca}$ channel agonists cromakalim and NS1619 in a concentration approximating that observed in CSF following FPI. Vasopressin so administered attenuated pial artery dilation to these $K$ channel activators under conditions of equivalent baseline diameter during non injury conditions (13 ± 6 and 23 ± 1 vs. 4 ± 1 and 10 ± 2% for cromakalim 10$,^-8$, 10$^-6$ M before and after vasopressin, respectively). Attenuated responses were fully restored when these agonists were coadministered with vasopressin and the vasopressin antagonist [l-(b-mercapto-b-b-cyclopentamethylene propionic acid) 2-(o-methyl)-Tyr-A VP] (MEA VP). Cromakalim and NS1619 induced pial artery dilation was attenuated following FPI and MEA VP preadministration partially prevented such impairment (13 ± 6 and 23 ± 1, sham control; 2 ± 1 and 5 ± 1, FPI; and 9 ± 1 and 15 ± 2%, FPI-MEA VP pretreated for responses to cromakalim 10$^-8$, 10$^-6$ M, respectively). These data show that vasopressin blunts $K_{ATP}$ and $K_{ca}$ channel mediated cerebrovasodilation. These data suggest that vasopressin contributes to impaired $K_{ATP}$ and $K_{ca}$ channel function after brain injury. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Disorders of the nervous system

Topic: Trauma

Keywords: Newborn; Cerebral circulation; $K^+$ Channel; Vasopressin; Brain injury

1. Introduction

Relaxation of blood vessels can be mediated by several mechanisms, including cGMP, cAMP, and $K^+$ channels [16]. Membrane potential of vascular muscle is a major determinant of vascular tone, and activity of $K^+$ channels is a major regulator of membrane potential [20]. Activation or opening of these channels increases $K^+$ efflux, thereby producing hyperpolarization of vascular muscle. Membrane hyperpolarization closes voltage dependent calcium channels and thereby causes relaxation of vascular muscle [19,20]. Direct measurements of membrane potential and $K^+$ current in vitro indicate that several different types of $K^+$ channels are present in cerebral blood vessels. In addition, a number of pharmacological studies using activators and inhibitors have provided functional evidence that $K^+$ channels, especially ATP sensitive $K^+$ ($K_{ATP}$) and calcium sensitive $K^+$ ($K_{ca}$) channels, regulate tone of cerebral blood vessels in vitro and in vivo [16]. While several recent studies have characterized the role of $K^+$ channels in cerebrovascular control under physiological conditions, less is known concerning their contributions under pathological conditions.

Traumatic brain injury is one of the major causes of morbidity, mortality, and pediatric intensive care unit admissions of children today [22,25]. Although the effects of traumatic brain injury have been well described for adult animal models [12,17,18,27], few have investigated these effects in the newborn. To reproduce some of the biomechanical aspects of closed head injury, fluid percussion brain injury (FPI) has been used in the adults of several species [17,18]. More recently, Prins et al. [21] characterized the effects of FPI on several parameters including mortality, intracranial pressure, and mean arterial
blood pressure in the developing and adult rat. Other earlier studies had compared the cerebral hemodynamic effects of FPI in newborn (1–5 days old) and juvenile (3–4 weeks old) pigs. For example, it was observed that pial vessels constricted more, and that regional cerebral blood flow decreased and remained depressed longer, in newborns than in juveniles [8].

Vasopressin contributes to the regulation of cerebral hemodynamics in the piglet [9–11]. Vasopressin is released into CSF by FPI and also contributes to impaired pial artery dilation to opioids such as dynorphin following such an insult in the piglet [5]. Dynorphin elicits pial vasodilation via activation of K$_{ATP}$ and K$_{ca}$ channels [3,24]. Since K$_{ATP}$ and K$_{ca}$ channel function is impaired after FPI [2,4], altered dilation to this opioid could relate to such impaired K$^+$ channel function. Interestingly, vasopressin has been observed to block the K$_{ATP}$ channel in porcine coronary artery smooth muscle cells [26]. However, vasopressin did not appear to have any direct effect on the K$_{ca}$ channel in the above studies [26].

Therefore, the present studies were designed to characterize the role of vasopressin in impaired pial artery dilation to activators of K$_{ATP}$ and K$_{ca}$ channels following FPI in the newborn pig.

2. Methods

Thirty-five newborn (1–5 days old, 1.3–2.1 kg) pigs of either sex (19 male, 16 female) were used in these experiments. All protocols were approved by the Institutional Animal Care and Use Committee. Animals were sedated with isoflurane (1–2 MAC). Anesthesia was maintained with α-chloralose (30–50 mg/kg, supplemented with 5 mg/kg per h iv). A catheter was inserted into a femoral artery to monitor blood pressure and to sample for blood gas tensions and pH. Drugs to maintain anesthesia were administered through a second catheter placed in a femoral vein. The trachea was cannulated, and the animals were mechanically ventilated with room air. A heating pad was used to maintain the animals at 37–39°C.

A cranial window was placed 0.5 cm from the bregma and the mid sagittal line in the parietal skull of these anesthetized animals. This window consisted of three parts: a stainless steel ring, a circular glass coverslip, and three ports consisting of 17-gauge hypodermic needles attached to three precut holes in the stainless steel ring. For placement, the dura was cut and retracted over the cut bone edge. The cranial window was placed in the opening and cemented in place with dental acrylic. The volume under the window was filled with a solution, similar to CSF, of the following composition (in mM): 3.0 KCl, 1.5 MgCl$_2$, 1.5 CaCl$_2$, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO$_3$. This artificial CSF was warmed to 37°C and had the following chemistry: pH 7.33, PCO2 46 mmHg, and PO2 43 mmHg, which was similar to that of endogenous CSF. Pial arterial vessels were observed with a dissecting microscope, a television camera mounted on the microscope, and a video output screen. Vascular diameter was measured with a video microscaler.

Methods for brain FPI have been described previously [27]. A device designed by the Medical College of Virginia was used. A small opening was made in the parietal skull contralateral to the cranial window, also 0.5 cm from begma. A metal shaft was sealed into the opening on top of intact dura. This shaft was connected to the transducer housing, which was in turn connected to the fluid percussion device. The device itself consisted of an acrylic plastic cylindrical reservoir 60 cm long, 4.5 cm in diameter, and 0.5 cm thick. One end of the device was connected to the transducer housing, whereas the other end had an acrylic plastic piston mounted on O-rings. The exposed end of the piston was covered with a rubber pad. The entire system was filled with 0.9% saline. The percussion device was supported by two brackets mounted on a platform. FPI was induced by striking the piston with a 4.8-kg pendulum. The intensity of the blow (usually 1.9–2.3 atm with a constant duration of 19–23 ms) was controlled by varying the height from which the pendulum was allowed to fall. The pressure pulse of the blow was recorded on a storage oscilloscope triggered photoelectrically by the fall of the pendulum. The amplitude of the pressure pulse was used to determine the intensity of the injury.

2.1. Protocol

Two types of pial arterial vessels, small arteries (resting diameter 120–160 μm) and arterioles (resting diameter 50–70 μm), were examined to determine whether segmental differences in the effects of FPI could be identified. Typically, 2–3 ml of CSF were flushed through the window over a 30-s period, and excess CSF was allowed to run off through one of the needle ports.

Five types of experiments were performed: (1) shamt control (n=6), (2) FPI (n=6), (3) FPI pretreated with MEA VP (n=6), (4) vasopressin with and without MEA VP pretreatment (n=12), and (5) time control (n=5).

In the FPI experiments, responses of arterial vessels to the synthetic K$_{ATP}$ channel agonist (-)-cromakalin (10$^{-8}$, 10$^{-6}$ M, Smith Kline Beechman), the endogenous K$_{ATP}$ channel activator calcitonin gene related peptide (CGRP 10$^{-8}$, 10$^{-6}$ M, Sigma Chemical) and the synthetic K$_{ca}$ channel agonist NS1619 (10$^{-8}$, 10$^{-6}$ M, Research Biochemicals Int.) were obtained before and 60 min after brain injury in the absence and presence of pretreatment 30 min prior to injury with [l-(β-mercapto-β,β-cyclopentamethylenely propionic acid) 2-(o-methyl)-Tyr-VP] (MEA VP 5 μg/kg i.v.). Sham control experiments were designed such that responses were obtained initially and then again 60 min later. Each of the drugs were applied in an ascending concentration manner. There was a period of
20 min after the highest concentration of one drug was washed off before a different drug was infused. The percent change in artery diameter values were calculated on the basis of the diameter measured in the control period for each drug before injury for preinjury (control) values, whereas the diameter present in the control period before the drug administration after injury was used for brain injury values.

In the vasopressin experiments, the responses of arterial vessels to cromakalim, CGRP and NS1619 were obtained in the absence of vasopressin, in the presence of vasopressin (40 and 400 pg/ml), concentrations observed in CSF after FPI, and in the presence of vasopressin and MEAVP (5 μg/kg i.v.). Because vasopressin is a vasodilator, U46619 (0.3 ng/ml), a vasoconstrictor, was coadministered with vasopressin to make certain that pial artery diameter was equivalent in the absence and presence of vasopressin. The vehicle for all agents was 0.9% saline, which had no effect on pial artery diameter. Confirmation of vasopressin receptor blockade was determined by comparing responses to this agonist before and after antagonist administration. Time control experiments were conducted in a separate series of animals and were designed to obtain responses to drugs initially and then 1 h later (designated as time 1 and time 2 in Table 1).

2.2. Statistical analysis

As described above, two different size pial arteries were measured for each intervention. In time control experiments, the reproducibility of such measurements was ±3 μm for vessels between 120 and 160 μm and ±1 μm for vessels between 50 and 70 μm in diameter. Pial arteriolar diameter and systemic arterial pressure values were analyzed using ANOVA for repeated measures. If the value was significant, the data were then analyzed by Fisher’s protected least significant difference test. An alpha level of P<0.05 was considered significant in all statistical tests. Values are represented as means±S.E. of the absolute values or percent changes from control values.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Time 1</th>
<th>Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small artery</td>
<td>Arteriole</td>
</tr>
<tr>
<td><strong>Cromakalim</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−10^3 M)</td>
<td>0</td>
<td>139±2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>159±3*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>171±3*</td>
</tr>
<tr>
<td><strong>CGRP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−10^3 M)</td>
<td>0</td>
<td>139±2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>156±3*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>174±2*</td>
</tr>
<tr>
<td><strong>NS1619</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−10^3 M)</td>
<td>0</td>
<td>132±2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>147±3*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>156±4*</td>
</tr>
</tbody>
</table>

*Values are μm±S.E.M., n=5.

3. Results

3.1. Effect of vasopressin on K<sub>ATP</sub> and K<sub>ca</sub> channel agonist induced pial artery dilation under non injury conditions

Cromakalim, CGRP, and NS1619 (10^−8, 10^−6 M) elicited reproducible pial small artery dilation (120–160 μm) and arteriole dilation (50–70 μm) (Table 1). In these experiments, there were 5 degrees of freedom for each agonist and the level of significance was P<0.001. Similar effects were observed in pial arterioles. Small artery Arteriole Small artery Arteriole

Since the concentration of substances detected in CSF reflect but may not be equivalent numerically to such changes within cells, the influence of a higher concentration of vasopressin (400 pg/ml) on K<sub>ATP</sub> and K<sub>ca</sub> channel agonist vasodilation was also considered. These experiments were also conducted under conditions of equivalent baseline diameter. Therefore, during coadministration of U46619 (0.3 ng/ml) with vasopressin (400 pg/ml), there was no net change in pial artery diameter (143±18 vs. 148±18 μm). Under such conditions of equivalent baseline diameter, vasopressin/U46619 coadministration with cromakalim, CGRP or NS1619 attenuated pial small artery dilation to these K<sup>+</sup> channel agonists (Fig. 1). Attenuated responses were fully restored when these agonists were coadministered with vasopressin and the vasopressin antagonist MEAVP (5 μg/kg, i.v.) (Fig. 1). In these statistical comparisons, there were 4 degrees of freedom for each agonist and the level of significance was P<0.001. Similar effects were observed in pial arterioles.

Since the concentration of substances detected in CSF reflect but may not be equivalent numerically to such changes within cells, the influence of a higher concentration of vasopressin (400 pg/ml) on K<sub>ATP</sub> and K<sub>ca</sub> channel agonist vasodilation was also considered. These experiments were also conducted under conditions of equivalent baseline diameter. Therefore, during coadministration of U46619 (0.3 ng/ml) with vasopressin (400 pg/ml), there was no net change in pial artery diameter (143±18 vs. 148±18 μm). Under such conditions of equivalent baseline diameter, vasopressin/U46619 coadministration with cromakalim, CGRP, and NS1619 further attenuated pial small artery dilation to these K<sup>+</sup> channel agonists (11±1 and 22±1 vs. 1±1 and 3±2% for cromakalim 10<sup>−8</sup>, 10<sup>−6</sup> M in the absence and presence of vasopressin, respectively n=6). Similar effects were observed in pial arterioles.

3.2. Role of vasopressin in impaired K<sub>ATP</sub> and K<sub>ca</sub> channel agonist induced pial artery dilation following FPI

Cromakalim, CGRP, and NS1619 induced pial small
artery dilation was attenuated within 1 h post FPI (Figs. 2–4). In animals pretreated with the vasopressin antagonist MEAVP, such impaired vasodilation was partially prevented, though responses were still attenuated compared to control (Figs. 2–4). Similar effects were observed in pial arterioles.

3.3. Confirmation of effective receptor blockade

Vascular responses to vasopressin were blocked by MEAVP (5 μg/kg i.v.) (9±1 and 15±1 vs. 1±1 and 2±1% for vasopressin 40 and 400 pg/ml before and after MEAVP, respectively, n=6). MEAVP did not have any significant effect on pial artery diameter.

3.3.1. Blood chemistry and intensity of injury

Blood chemistry values were obtained at the beginning and the end of all experiments. These values were: 7.46±0.02, 34±5, and 95±9 mmHg vs. 7.45±0.02, 35±6, and 94±8 mmHg for pH, $P_{CO_2}$, and $P_O_2$, respectively before and after injury. Administration of MEAVP did not
NS1619 induced pial artery dilation to a greater extent than that observed with coadministration of vasopressin (40 pg/ml). Although the precise concentration at the receptor level is uncertain, the lower concentration (e.g., 40 pg/ml) is approximately the one observed for vasopressin in cortical periarachnoid CSF 1 h after FPI [5]. Since CSF concentrations reflect but are not equivalent to changes in substance concentration at the receptor level (where the effective concentration presumably is higher), these data support the functional significance of the interaction between vasopressin and K⁺ channel activators.

Another series of experiments were designed to further investigate the functional significance of the above described modulatory role of vasopressin in K⁺ channel mediated vasodilation. In particular, cromakalim, CGRP, and NS1619 induced pial artery dilation was attenuated within 1 h of FPI, consistent with previous studies [2,4]. New data from the present study show that MEAVP partially prevented such diminished K⁺ channel agonist vasodilation post insult. These data suggest that vasopressin contributes to K⁺ and K⁺ channel function impairment observed following FPI. Previous studies showing that MEAVP attenuated pial artery vasoconstriction induced by FPI (5) indicate that vasopressin contributes to impaired cerebral hemodynamics following brain injury. In that systemic MEAVP blocked the vascular action of topical vasopressin without affecting the response to other substances (10), these data indicate that this antagonist was selective for vasopressin and that it crosses the blood brain barrier in sufficient quantity.

Cellular sites of origin for vasopressin detected in CSF in previous studies [5] include neurons, glia, vascular smooth muscle, and endothelial cells. The present experimental design, however, does not allow any conclusion to be drawn with respect to cellular sites of origin for vasopressin. Because MEAVP had no significant effect on baseline pial artery diameter, vasopressin probably has minimal contribution to cerebrovascular tone during physiologic conditions. In that pial small arteries exhibited about the same percentage decrease in responsiveness to K⁺ channel activators following FPI as that observed with pial arterioles, these data also suggest that there are probably minimal regional segmental vascular differences in altered K⁺ channel agonist activity following FPI.

Previous studies have investigated the selectivity of the agents used as probes for K⁺ and K⁺ channel activation induced pial artery dilation. Cromakalim induced pial artery dilation has been observed to be blocked by glibenclamide and unchanged by iberiotoxin, K⁺ and K⁺ channel antagonists respectively [7]. Conversely, NS1619 induced pial artery dilation was blocked by iberiotoxin and unchanged by glibenclamide [3,4,7]. These data suggest that cromakalim and NS1619 are selective K⁺ and K⁺ channel agonists in the piglet cerebral circulation. Pial arteries have been shown to be innervated by CGRP containing nerve fibers [13]. CGRP produces hyperpolari-

4. Discussion

Results of the present study show that coadministration of vasopressin with cromakalim, CGRP, or NS1619 attenuated vasodilatation to these K⁺ channel agonists in a non injury state. Because vasopressin had a vascular effect of its own, U46619 was coadministered with vasopressin in a concentration that resulted in a no net change in pial artery diameter. Therefore, vasopressin, in effect, was coadministered with K⁺ channel activators under conditions of equivalent baseline diameter. Because previous studies have shown that U46619, in concentrations higher than that used in the present study (1 vs. 0.3 ng/ml), did not have any effect on pial dilation to cromakalim and CGRP (2), vasopressin alone appears to exert these inhibitory effects. Similar unpublished studies have observed that U46619 (1 ng/ml) did not affect NS1619 induced pial artery dilation. Additional experiments were performed to determine if there was a dose response relationship to this inhibitory action of vasopressin on K⁺ channel agonists. Results of these studies show that vasopressin (400 pg/ml) inhibited cromakalim, CGRP, and
zation of cerebral vascular muscle in vitro [23], and cross selectivity experiments have similarly been performed supportive of its selectivity for the $K_{\text{ATP}}$ channel in the piglet [7]. Inclusion of data for CGRP in the present study, therefore, lends physiologic functional perspective to results indicative of vasopressin’s modulatory role in $K_{\text{ATP}}$ channel vascular function. However, it has also been observed that NS1619 may additionally possess calcium channel antagonistic activity and, therefore, may not be useful as a probe for $K_{\text{ca}}$ channel activation [14]. In contrast, recent observations in the piglet show that vasoconstrictor responses to the calcium channel agonist Bay K8644 were unchanged in the presence of NS1619 [3]. These results suggest that NS1619 has no calcium channel blocking activity and, therefore, may be considered to be selective for activation of $K_{\text{ca}}$ channels in the newborn pig.

Previous studies have observed that vasopressin is released into CSF and contributes to altered dilation to the opioid dynorphin following FPI in the newborn pig [5]. Results of the present study extend the latter observations to indicate that vasopressin also modulates $K_{\text{ATP}}$ and $K_{\text{ca}}$ channel agonist mediated vascular activity following FPI, suggestive of more distal signal transduction impairment post insult. Although the mechanism for such vasopressinergic modulation of $K^+$ channel function following FPI is uncertain, one possibility could relate to a change in the vascular response to vasopressin following FPI, resulting in physiologic antagonism of $K^+$ channel induced vasodilation. Specifically, vasopressin reverses from a dilator to a vasoconstrictor following FPI [6]. Such vasoconstriction could, therefore, oppose the ability of $K_{\text{ATP}}$ and $K_{\text{ca}}$ channel agonists to vasodilate.

Alternatively, vasopressin could also indirectly alter $K^+$ channel agonist vasodilation. Support for this position comes from the observation that vasopressin impairs $K_{\text{ATP}}$ and $K_{\text{ca}}$ channel mediated vasodilation even in the absence of traumatic brain injury (and under conditions of equivalent baseline diameter). While cellular studies have shown a similar modulatory role of vasopressin for the $K_{\text{ATP}}$ channel in isolated pig coronary artery smooth muscle cells [26], results of the present study are the first to demonstrate such a relationship between vasopressin and the $K_{\text{ca}}$ channel. In unrelated studies, endothelin-1 has been observed to contribute to $K_{\text{ATP}}$ channel mediated vasodilator impairment following FPI via a protein kinase C (PKC) dependent generation of superoxide anion [1,15]. The role of PKC activation in vasopressin induced impairment of $K_{\text{ATP}}$ and $K_{\text{ca}}$ channel mediated vasodilation following FPI is uncertain. Additionally, brain injury could alter the number or binding of $K^+$ channels available for activation, the degree of hyperpolarization that subsequently occurs or the ultimate response to hyperpolarization itself.

In conclusion, results of the present study show that vasopressin blunts $K_{\text{ATP}}$ and $K_{\text{ca}}$ channel mediated cerebrovasodilation. These data suggest that vasopressin contributes to impaired $K_{\text{ATP}}$ and $K_{\text{ca}}$ channel function after brain injury.

Acknowledgements

The authors thank Miriam Kulkarni and John Ross for technical assistance with performance of the experiments. This research was supported by grants from the National Institutes of Health.

References


