Vasopressin-Induced Protein Kinase C–Dependent Superoxide Generation Contributes to ATP-Sensitive Potassium Channel but Not Calcium-Sensitive Potassium Channel Function Impairment After Brain Injury

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Background and Purpose—Pial artery dilation in response to activators of the ATP-sensitive K⁺ (K_ATP) and calcium-sensitive K⁺ (K_Ca) channels is impaired after fluid percussion brain injury (FPI). Vasopressin, when coadministered with the K_ATP and K_Ca channel agonists cromakalim and NS1619 in a concentration approximating that observed in cerebrospinal fluid (CSF) after FPI, blunted K_ATP and K_Ca channel–mediated vasodilation. Vasopressin also contributes to impaired K_ATP and K_Ca channel vasodilation after FPI. In addition, protein kinase C (PKC) activation generates superoxide anion (O₂⁻), which in turn contributes to K_ATP channel impairment after FPI. We tested whether vasopressin generates O₂⁻ in a protein kinase C (PKC)-dependent manner, which could link vasopressin release to impaired K_ATP and K_Ca channel–induced pial artery dilation after FPI.

Methods—Injury of moderate severity (1.9 to 2.1 atm) was produced with the lateral FPI technique in anesthetized newborn pigs equipped with a closed cranial window. Superoxide dismutase–inhibitable nitroblue tetrazolium (NBT) reduction was determined as an index of O₂⁻ generation.

Results—Under sham injury conditions, topical vasopressin (40 pg/mL, the concentration present in CSF after FPI) increased superoxide dismutase–inhibitable NBT reduction from 161 to 2364 pmol/mm². Chelerythrine (10⁻⁷ mol/L, a PKC inhibitor) blunted such NBT reduction (161 to 962 pmol/mm²), whereas the vasopressin antagonist l-(β-mercapto-β,β-cyclopentamethylene propionic acid)2-(o-methyl)-Tyr-arginine vasopressin (MEAVP) blocked NBT reduction. Chelerythrine and MEAVP also blunted the NBT reduction observed after FPI (161 to 15±1, 1±1 to 4±1, and 1±1 to 5±1 pmol/mm² for sham-, chelerythrine-, and MEAVP-treated animals, respectively). Under sham injury conditions, vasopressin (40 pg/mL) coadministered with cromakalim or NS1619 blunted dilation in response to these K⁺ channel agonists, whereas chelerythrine partially restored such impaired vasodilation for cromakalim but not NS1619. Cromakalim- and NS1619-induced pial artery dilation also was blunted after FPI. MEAVP partially protected dilation to both K⁺ channel agonists after FPI, whereas chelerythrine did so for only cromakalim responses (for cromakalim at 10⁻⁸ and 10⁻⁶ mol/L, 13±1% and 23±1%, 2±1% and 5±1%, 9±1% and 15±2%, and 9±1% and 16±2% for sham-, FPI-, FPI-MEAVP-, and FPI-chelerythrine–pretreated animals, respectively).

Conclusions—These data show that vasopressin, in concentrations present in CSF after FPI, increased O₂⁻ production in a PKC-dependent manner and contributes to such production after FPI. These data show that vasopressin contributes to K_ATP but not K_Ca channel function impairment in a PKC-dependent manner after FPI and suggest that vasopressin contributes to K_Ca channel function impairment after FPI via a mechanism independent of PKC activation. (Stroke. 2001;32:1408-1414.)

Key Words: brain injuries ■ cerebral circulation ■ free radicals ■ newborn ■ potassium channels ■ vasopressin

Clinical investigations have suggested that a major part of neural damage in traumatic brain injury may be secondary to decreased cerebral blood flow.¹⁻³ It is now generally accepted that secondary events account for a significant component of the damage that occurs after brain injury. Such secondary factors include the generation of oxygen free radicals, bioenergetic dysfunction, ion changes, and neurotransmitters and their modulator release.⁴⁻⁶ In particular, the regulation of vascular tone and vasoactive responsiveness after the initial insult is thought to contribute to stroke pathogenesis. Relaxation of blood vessels can be mediated by several mechanisms, including cGMP, cAMP, and K⁺ channels.⁷ Membrane potential of vascular muscle is a major determi-
nant of vascular tone, and activity of K⁺ channels is a major regulator of membrane potential.8,9 A number of pharmacological studies with activators and inhibitors have provided functional evidence that K⁺ channels, especially ATP-sensitive K⁺ (K_ATP) and calcium-sensitive K⁺ (K_Ca) channels, regulate the tone of cerebral blood vessels in vitro and in vivo.7 Although 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 complications, death, and pediatric intensive care unit admissions of children today.10,11 Although the effects of traumatic brain injury have been well described for adult animal models,12–15 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 and newborns of several species.13–16 Vasopressin elicits pial artery dilation and contributes to the regulation of cerebral hemodynamics in the piglet.17–19 Vasopressin is released into cerebrospinal fluid (CSF) by FPI and contributes to impaired pial artery dilation in response to opioids such as dynorphin after such an insult in the piglet.20 Dynorphin elicits pial vasodilation via the activation of K_ATP and K_Ca channels.21,22 Because K_ATP and K_Ca channel functions are impaired after FPI,23,24 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.25 However, vasopressin did not appear to have a direct effect on the K_Ca channel in the studies just cited.25 More recent studies, though, have shown that vasopressin does modulate both K_ATP and K_Ca channel agonist–induced pial artery dilation and contributes to impairment of both K⁺ channel subtypes after FPI in the newborn pig.26 The mechanism by which vasopressin might contribute to K_ATP and K_Ca channel function impairment after FPI is uncertain. In addition, although the activation of protein kinase C (PKC) has been observed to generate superoxide anion (O₂⁻), which in turn contributes to K_ATP channel function impairment after FPI,27 such a role for O₂⁻ in K_Ca channel impairment after this insult is unknown.

We therefore designed the present study to characterize mechanisms involved in impaired cerebrovascular control contributory to secondary ischemia after traumatic brain injury. Specifically, this study was designed to determine whether vasopressin generates O₂⁻ in a PKC-dependent manner, which could link vasopressin release to impaired K_ATP and K_Ca channel–induced pial artery dilation after FPI.

### Materials and Methods

Newborn (1 to 5 days old, 1.3 to 2.1 kg) pigs of either sex were used in these experiments. All protocols were approved by the Institutional Animal Care and Use Committee. Animals were sedated with isoflurane (1 to 2 minimum alveolar concentration). Anesthesia was maintained with α-chloralose (30 to 50 mg/kg, supplemented with 5 mg·kg⁻¹·h⁻¹ IV). The trachea was cannulated, and the animals were mechanically ventilated with room air. A heating pad was used to maintain the animals at 37° to 39°C. The cranial window technique was used to visualize pial arteries, as described previously.16

Methods for brain FPI have been described previously.13 A small opening was made in the parietal skull contralateral to the cranial window for fluid coupling of the injury device to the animal. The pressure pulse of the insult 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.

### Protocol

Two types of pial arterial vessels, small arteries (resting diameter 120 to 160 μm) and arterioles (resting diameter 50 to 70 μm), were examined to determine whether segmental differences in the effects of FPI on K_ATP and K_Ca channel agonist pial dilation could be identified.

Four major types of protocols were performed. In protocol 1, we determined O₂⁻ generation induced by vasopressin (n = 7) (1) in the presence of the PKC inhibitor chelerythrine, n = 7; and (2) in the presence of [l-(β-mercapto-β,β-cyclopentamethylene propionic acid)-2-(α-methyl)-Tyrv-arginine vasopressin (MEAVP), a vasopressin receptor antagonist, n = 7) and induced by FPI in (1) untreated animals (n = 7), (2) in chelerythrine-pretreated animals (n = 7), and (3) in MEAVP-pretreated animals (n = 7). In protocol 2, we determined K_ATP/K_Ca vascular responses induced by vasopressin (n = 1) sham control, n = 6; (2) coadministered with vasopressin, n = 6; (3) coadministered with staurosporine, n = 6; and (4) coadministered with chelerythrine, n = 6) and induced by FPI (1) sham control, n = 6; (2) after FPI, n = 6; (3) in chelerythrine-pretreated animals, n = 6; and (4) in MEAVP-pretreated animals, n = 6). In protocol 3, we determined O₂⁻ generation with K_ATP agonist selectivity and FPI (n = 6).

In the first 3 series of experiments designed to investigate the generation of O₂⁻, lysine vasopressin, the vasopressin isofrom present in the pig (40 pg/mL; Sigma Chemical Co), was applied to the cerebral cortex for 20 minutes in either the absence or presence of the PKC inhibitor chelerythrine (10⁻⁷ mol/L; Sigma Chemical Co) or the vasopressin antagonist MEAVP (5 μg/kg IV; Sigma Chemical Co). In the next 3 series of experiments, the generation of O₂⁻ 1 hour after FPI was investigated in the absence and presence of chelerythrine or MEAVP. In these experiments, chelerythrine or MEAVP was administered 30 minutes before FPI. Chelerythrine was kept in constant contact with the cerebral cortex for the duration of the experiment. Because the techniques for measurement of O₂⁻ generation (see later) involves the placement of detection solutions on the cerebral cortex for 20 minutes, such measurement in fact reflects O₂⁻ generation during the first 20-minute period 1 hour after FPI. The efficacy of chelerythrine as a PKC inhibitor was ascertained by determining the O₂⁻ generation induced by phorbol-12,13-dibutyrate, a PKC activator, in the absence and presence of chelerythrine.

In the vasopressin experiments, the responses of arterial vessels to the synthetic K_ATP channel agonist (~)-cromakalim (10⁻⁸ and 10⁻⁶ mol/L; SmithKline Beecham), the endogenous K_ATP channel activator calcitonin gene–related peptide (CGRP) (10⁻⁷ and 10⁻⁵ mol/L; Sigma Chemical Co), and the synthetic K_Ca channel activator NS1619 (10⁻⁸ and 10⁻⁶ mol/L; Sigma Chemical Co) were obtained in the absence of vasopressin, in the presence of vasopressin (40 pg/mL, the concentration observed in CSF after FPI), in the presence of vasopressin and the PKC inhibitor staurosporine, and in the presence of vasopressin and the PKC inhibitor chelerythrine (10⁻⁷ mol/L; Sigma Chemical Co). Because vasopressin is a vasodilator, U46619 (0.3 ng/mL), a vasoconstrictor, was coadministered with vasopressin to ensure pial artery diameter was equivalent in the absence and presence of vasopressin.

In the FPI experiments, responses of arterial vessels to cromakalim, CGRP, and NS1619 were obtained before and 60 minutes after brain injury in the absence and presence of pretreatment 30 minutes before injury with MEAVP (5 μg/kg IV) or chelerythrine (10⁻⁷ mol/L). Sham control experiments were designed such that responses were obtained initially and then again 60 minutes later.
Each of the drugs was applied in an ascending-concentration manner. There was a period of 20 minutes after the highest concentration of 1 drug was washed off before a different drug was infused.

In the activated oxygen experiments, vascular responses to NS1619 were obtained before and 20 minutes after exposure to an activated oxygen-generating system. The latter system consisted of 0.2 U/mL xanthine oxidase, 0.6 mmol/L hypoxanthine, and 0.02 mmol/L FeCl3 administered repeatedly at 5-minute intervals during a 20-minute period. Piglets treated with the inactivated oxygen-generating system were initially treated with oxyxurinol (50 mg/kg 30 minutes before experimentation) to inhibit endogenous xanthine oxidase. They then were treated as described earlier, but the xanthine oxidase was replaced with one that had been boiled for 30 minutes to inactivate the enzyme.

In the K_Ca/K_ATP channel agonist selectivity after FPI experiments, responses to cromakalin and CGRP were obtained in the absence and presence of glibenclamide (10^{-6} mol/L), a K_ATP channel antagonist, or iberiotoxin (10^{-7} mol/L), a K_Ca channel antagonist. Conversely, NS1619-induced vasodilation after FPI was observed in the absence and presence of either glibenclamide or iberiotoxin.

**O_2^-** Analysis

Superoxide dismutase (SOD)-inhibitable nitroblue tetrazolium (NBT) reduction was determined as an index of O_2^- generation, as previously described. Such reduction was determined by placing NBT (2.4 mmol/L; Sigma Chemical Co) dissolved in artificial CSF under the other window 1 hour after FPI. Because such solutions remained on the surface for 20 minutes, data are quantified as picomoles of NBT reduced for 20 minutes. Two windows were placed contralateral to the adapter for the induction of FPI for these experiments.

**Statistical Analysis**

Pial arteriolar diameter, systemic arterial pressure, and the NBT-reduced values were analyzed using ANOVA for repeated measures. If the value was significant, the data were analyzed with Fisher’s protected least significant difference test. An α level of P<0.05 was considered significant in all statistical tests. Values are represented as mean±SEM of the absolute values or percent changes from control values.

**Results**

**Role of PKC Activation in Vasopressin-Induced O_2^- Generation Under Sham Injury and Brain Injury Conditions**

Topical vasopressin (40 pg/mL, the concentration present in CSF after FPI) applied to the cerebral cortical surface of sham injured animals increased SOD-inhibitable NBT reduction (Figure 1A). Such NBT reduction by vasopressin was blunted by chelerythrine (10^{-7} mol/L) and blocked by the vasopressin antagonist MEAVP (5 μg/kg IV) (Figure 1A). Under brain injury conditions, SOD-inhibitable NBT reduction was increased 1 hour after FPI (Figure 1B). Such enhanced NBT reduction after FPI was blunted by both MEAVP and chelerythrine (Figure 1B). Under sham injury conditions, topical phorbol-12,13-dibutyrate (10^{-6} mol/L), a PKC activator, increased SOD-inhibitable NBT reduction from 1±3 to 37±5 pmol/mm^2, which was blocked by chelerythrine coadministration (1±1 to 3±2 pmol/mm^2).

**Role of PKC Activation in Vasopressin Modulation of K_ATP and K_Ca Channel Agonist-Induced Pial Artery Dilation Under Sham Injury Conditions**

Cromakalin, CGRP, and NS1619 (10^{-8} and 10^{-6} mol/L) elicited reproducible pial small artery (120 to 160 μm) and arteriole (50 to 70 μm) vasodilation (data not shown). Vasopressin (40 pg/mL) increased pial small artery diameter from 149±5 to 163±7 μm (n=6). During the coadministration of U46619 (0.3 ng/mL) with vasopressin (40 pg/mL), there was no net change in pial artery diameter (149±12 versus 153±13 μm). Under such conditions of equivalent baseline diameter, vasopressin/U46619 coadministered with cromakalin, CGRP, or NS1619 attenuated pial small artery dilation in response to these K^+ channel agonists (Figure 2). Attenuated responses were partially restored when these agonists were coadministered with vasopressin and either staurosporine (10^{-7} mol/L) or chelerythrine (10^{-7} mol/L), both of which are PKC inhibitors (Figure 2). However, this was not found for NS1619. Similar effects were observed in pial arterioles (data not shown).

**Role of PKC Activation in Vasopressin Impairment of K_ATP and K_Ca Channel Agonist-Induced Pial Artery Dilation After FPI**

Cromakalin-, CGRP-, and NS1619-induced pial small artery dilation was attenuated within 1 hour after FPI (Figure 3). In animals pretreated with the vasopressin antagonist MEAVP or the PKC inhibitor chelerythrine, such impaired vasodila-
tion was partially prevented, although responses were still attenuated compared with control (Figure 3). Similar effects were observed in pial arterioles (data not shown). Pial small artery diameter was reduced from 143 ± 7 to 124 ± 5 and pial arteriole diameter was reduced from 65 ± 3 to 50 ± 3 μm within 1 hour of FPI (n = 6).

**Influence of a Xanthine Oxidase O$_2^-$-Generating System on K$_{Ca}$ Channel Agonist–Induced Pial Artery Dilation Under Noninjury Conditions**

NS1619-induced pial small artery dilation was attenuated after exposure of the cerebral cortical surface to the active oxygen-generating system (Figure 4). The inactive oxygen-generating system, however, had no effect on pial small artery dilation to NS1619 (data not shown). Similar effects were observed in pial arterioles (data not shown).

**Confirmation of Effective Receptor Blockade**

Vascular responses to vasopressin were blocked by MEAVP (5 μg/kg IV) (10 ± 1% versus 1 ± 1% for vasopressin 40 pg/mL before and after MEAVP, respectively; n = 6). MEAVP did not have a significant effect on pial artery diameter.

**Confirmation of K$_{ATP}$ and K$_{Ca}$ Channel Agonist Specificity After FPI**

Cromakalim- and CGRP-induced pial artery dilation was blocked by glibenclamide (10$^{-6}$ mol/L) and unchanged by iberiotoxin (10$^{-7}$ mol/L) after FPI (for cromakalim 10$^{-8}$ and 10$^{-6}$ mol/L, 1 ± 1% and 4 ± 1% for FPI, 0 ± 1% and 0 ± 1% for FPI-glibenclamide, and 1 ± 1% and 5 ± 1% for FPI-iberiotoxin, respectively; n = 6). Similarly, NS1619-induced pial artery dilation was blocked by iberiotoxin (10$^{-7}$ mol/L) and unchanged by glibenclamide (10$^{-6}$ mol/L) after FPI (for NS1619 10$^{-8}$ and 10$^{-6}$ mol/L, 3 ± 1% and 5 ± 1% for FPI, 0 ± 1% and 0 ± 1% for FPI-iberiotoxin, and 3 ± 1% and 6 ± 1% for FPI-glibenclamide, respectively; n = 6). Glibenclamide and iberiotoxin had no effect on pial artery diameter after FPI.

**Blood Chemistry and Intensity of Injury**

Blood chemistry values were obtained at the beginning and the end of all experiments; these values were 7.45 ± 0.01, 35 ± 5, and 94 ± 6 mm Hg versus 7.44 ± 0.02, 36 ± 5, and 93 ± 6 mm Hg for pH, P$_{CO_2}$, and P$_{O_2}$, respectively, before and after injury. The administration of MEAVP did not significantly affect blood chemistry values. The amplitude of the
pressure pulse used as an index of injury intensity was 2.0±0.1 atm.

Discussion

The results of the present study show that under sham injury conditions, the topical administration of vasopressin, in a concentration observed in CSF after FPI, resulted in increased SOD-inhibitable NBT reduction by newborn pig brain, indicating that O$_2^-$ was generated. Because chelerythrine blunted such elevation in SOD-inhibitable NBT reduction by vasopressin, these data indicate that the activation of PKC contributes to O$_2^-$ generation by vasopressin. Moreover, the vasopressin antagonist MEAVP blocked such NBT reduction, indicating that vasopressin generates O$_2^-$ in a selective manner. In addition, both chelerythrine and MEAVP blunted brain injury–induced elevated SOD-inhibitable NBT reduction. Previously, FPI has been observed to be associated with the generation of O$_2^-$ on the piglet cerebral cortical surface.27,28 In those studies, it was also observed that FPI caused the release of endothelin-1 into CSF, which in turn contributed to the generation of O$_2^-$ after injury through the activation of PKC.27,28 More recent studies have shown that the opioid nociceptin/orphanin FQ contributes to O$_2^-$ generation after this insult in a mechanism dependent on the activation of cyclooxygenase.29 Results of the present study extend the latter observations to indicate that several vasoactive substances released into CSF after brain injury have the ability to contribute to O$_2^-$ generation after this insult via separate signal transduction pathways. In addition, because chelerythrine blocked the NBT reduction associated with topical administration of the PKC activator phorbol-12,13-dibutyrate, this probe appears to be an efficacious PKC inhibitor, strengthening the conclusions of the present study. However, it should be noted that concerns related to the accuracy of the NBT assay have been raised.30 Such concerns, related in large part to the specificity of NBT reduction as an index of O$_2^-$, have been limited by recent piglet studies supportive of such specificity.31

The cerebrovascular consequences of free radical production are not fully understood. It has been suggested that O$_2^-$ could be involved in irreversible vascular damage, delayed hypoperfusion, and edema produced by cerebral ischemia/reperfusion.32 The topical application of a xanthine/xanthine oxidase–activated oxygen-generating system, severe hypertension, the topical application of arachidonic acid, and fluid percussion brain injury cause morphological, functional, and biochemical cerebral artery abnormalities, which include reduced responsiveness to vasoconstrictor and vasodilator stimuli.15,28,33-35 Superoxide anion and species derived from it, such as hydrogen peroxide and hydroxyl radical, appear to mediate these abnormalities.28,35 The intracellular generation of O$_2^-$ or other species could alter the structure and/or production of nucleotides, second messengers, receptors, and membranes, and the movement of superoxide out of the cell through anion channels could result in high concentrations of activated oxygen species at cell surfaces, including the endothelium. More important, current concepts point toward the significant contribution to damage by the reaction of superoxide with nitric oxide to form the highly reactive prooxidant peroxynitrite.36,37 The latter species, and not O$_2^-$, is currently thought to be the more direct mediator of damage.

Results of the present study also show that the coadministration of vasopressin with cromakalim, CGRP, or NS1619 attenuated pial small artery vasodilation in response to these K$^+$ channel agonists in a sham injury state similar to recent observations.26 Because vasopressin had a vascular effect of its own, U46619 was coadministered with vasopressin in a concentration that resulted in no net change in pial artery diameter. Therefore, vasopressin, in effect, was coadministered with K$^+$ channel activators under conditions of equivalent baseline diameter. Although the precise concentration at the receptor level is uncertain, the concentration used in this study (eg, 40 pg/mL) is approximately that observed for vasopressin in cortical periarachnoid CSF 1 hour after FPI.20 Because 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. Because vasopressin inhibited dilation to K$^+$ channel agonists similarly in both pial small arteries and arterioles, there appears to be minimal regional vascular differences in this observed interaction.

Additional results of the present study show that the PKC inhibitors staurosporine and chelerythrine partially restored decremented pial artery dilation responses to K$\text{ATP}$, but not K$\text{Ca}$, channel agonists observed in the presence of coadministered vasopressin under sham injury conditions. These data suggest that activation of this signal transduction pathway by vasopressin contributes to such impaired K$\text{ATP}$ channel vasoreactivity. This conclusion is strengthened by the similar observation of partial restoration of vascular reactivity with 2 different PKC inhibitors. These data also indicate, however, that vasopressin contributes to the modulation of K$\text{Ca}$ channel–mediated pial artery dilation via a mechanism independent of PKC activation.

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 hour of FPI, which is consistent with previous studies.23,24 MEAVP partially prevented such diminished K$^+$ channel agonist vasodilation after the insult, similar to recent observations.26 These data suggest that vasopressin contributes to K$\text{ATP}$ and K$\text{Ca}$ channel function impairment after FPI. Previous studies showing that MEAVP attenuated pial artery vasoconstriction induced by FPI20 indicate that vasopressin contributes to impaired cerebral hemodynamics after brain injury. In that systemic MEAVP blocked the vascular action of topical vasopressin without affecting the response to other substances,18 these data indicate that this antagonist was selective for vasopressin and that it crosses the blood-brain barrier in sufficient quantity. New data in the present study show that chelerythrine partially prevented cromakalim and CGRP, but not NS1619, dilator impairment after FPI. These data suggest that vasopressin contributes to K$\text{ATP}$ channel function impairment after FPI via activation of PKC. Previous studies have
shown that PKC activation generates O$_2^-$, which then impairs K$_{ATP}$ channel agonist–mediated vasodilation. Taken together, these data suggest that vasopressin activates PKC, which in turn generates O$_2^-$ to impair K$_{ATP}$ channel function after FPI. On the other hand, the lack of protection for NS1619-induced pial artery dilation after FPI with chelerythrine could relate to (1) an inability of O$_2^-$ to impair K$_{Ca}$ channel function or (2) a mechanism by which vasopressin generates O$_2^-$ to impair K$_{Ca}$ channel function independent of PKC activation. Experiments in the present study showing that the topical application of a xanthine-based activated oxygen-generating system to the cerebral cortical surface blunted NS1619-induced pial artery dilation support the latter possibility. These data, then, indicated that O$_2^-$ generation after FPI contributes to K$_{Ca}$ channel impairment after this insult but that the mechanism for such O$_2^-$ generation and impairment of K$_{Ca}$ channel function is independent of PKC activation. These data therefore suggest that there are fundamental differences in the mechanism by which O$_2^-$ interacts with and impairs K$_{ATP}$ and K$_{Ca}$ channel function.

Previous studies have investigated the selectivity of the agents used as probes for K$_{ATP}$ and K$_{Ca}$ channel activation–induced pial artery dilation. Cromakalim-induced pial artery dilation has been observed to be blocked by glibenclamide and unchanged by iberiotoxin, K$_{ATP}$, and K$_{Ca}$ channel antagonists, respectively. Conversely, NS1619-induced pial artery dilation was blocked by iberiotoxin and unchanged by glibenclamide. These data suggest that cromakalim and NS1619 are selective K$_{ATP}$ and K$_{Ca}$ channel agonists in the piglet cerebral circulation. Pial arteries have been shown to be innervated by CGRP-containing nerve fibers. CGRP produces hyperpolarization of cerebral vascular muscle in vitro, and cross-selectivity experiments have similarly been performed supportive of its selectivity for the K$_{ATP}$ channel in the piglet. The inclusion of data for CGRP in the present study therefore lends physiological functional perspective to results indicative of the modulatory role of vasopressin in K$_{ATP}$ channel vascular function. However, it has also been observed that NS1619 may possess calcium channel antagonistic activity and therefore may not be useful as a probe for K$_{Ca}$ channel activation. In contrast, recent observations in the piglet show that vasocostrictror responses to the calcium channel agonist Bay K 8644 were unchanged in the presence of NS1619. These results suggest that NS1619 has no calcium channel–blocking activity and therefore may be considered to be selective for the activation of K$_{Ca}$ channels in the newborn pig. Finally, because it is possible that the above agonist specificity could be lost under brain injury conditions, additional selectivity experiments were performed under such brain injury conditions. Results of these studies show that glibenclamide blocked cromakalim- and CGRP-induced pial artery dilation, whereas such responses were unchanged by iberiotoxin. Conversely, NS1619 was blocked by iberiotoxin but unchanged by glibenclamide. These data therefore indicate that such pharmacologic specificity was obtained after FPI as well.

Previous studies have shown that vasopressin is released into CSF and contributes to altered dilation to the opioid dynorphin after FPI in the newborn pig. Results of the present study extend the latter observations to indicate that vasopressin also modulates K$_{ATP}$ and K$_{Ca}$ channel agonist–mediated vascular activity after FPI, suggestive of more distal signal transduction impairment after the insult. Results of this study also suggest that O$_2^-$ generation via PKC activation contributes to K$_{ATP}$, but not K$_{Ca}$, channel function impairment after FPI. Alternatively, vasopressin modulation of K$_{ATP}$ and K$_{Ca}$ channel function after FPI could relate to a physiological antagonism of K$^+$ channel–induced vasodilation. Specifically, vasopressin reverses from a dilator to a vasoconstrictor after FPI. Such vasoconstriction could therefore oppose the ability of K$_{ATP}$ and K$_{Ca}$ channel agonists to vasodilate. In addition, 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.

Placing the observations of this study in physiological perspective, results of the present study suggest that a neurohormone released in response to a primary insult may contribute to secondary mechanisms important in the regulation of cerebrovascular tone. Such observations are novel in that they link several factors previously implicated in secondary damage after brain injury (neurohormones, oxygen free radicals) to K$^+$ channel function impairment as contributory to ischemic stroke sequelae. In particular, cerebral ischemia has been observed to impair the dilation of cerebral arterioles in response to K$_{ATP}$ channel activators. For example, global cerebral ischemia has been observed to impair responses to CGRP and aprikalim in piglets. Also, dilation of pial arteries in response to RPS2891, a K$_{ATP}$ channel activator, was observed to be impaired in diabetic rats, whereas basilar artery dilation in response to aprikalim was blunted in stroke-prone spontaneously hypertensive rats. Because K$^+$ channels regulate the tone of cerebral blood vessels, impaired vascular responsiveness to activators of these channels after brain injury suggests the contribution of such signal transduction mechanisms to stroke pathogenesis. It is presently uncertain, however, whether the observations made in the present study can be translated to the adult state or if they are unique to the perinate.

In conclusion, results of the present study show that vasopressin, in concentrations present in CSF after FPI, increased O$_2^-$ production in a PKC-dependent manner and contributes to such production after FPI. These data show that vasopressin contributes to K$_{ATP}$, but not K$_{Ca}$, channel function impairment in a PKC-dependent manner after FPI. These data suggest that vasopressin contributes to K$_{Ca}$ channel function impairment after FPI via a mechanism independent of PKC activation.

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