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Influence of Sex and ERK MAPK on the Pressure Reactivity Index in Newborn Piglets After Fluid Percussion Injury

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Abstract

Greater impairment in autoregulation is seen in male versus female piglets following fluid percussion injury (FPI). This is partially mediated by a greater upregulation of extracellular signal-related kinase mitogen-activated protein kinase (ERK MAPK). We hypothesized that these trends would be reflected by the pressure reactivity index (PRx), a clinical measure of autoregulation. We further hypothesized that PRx values would correlate well with pial artery dilatory responses to hypotension. Male and female piglets were subjected to FPI and treated with a vehicle or ERK MAPK antagonist U 0126 (1 mg/kg IV) 30 min post-injury. FPI led to upregulation of CSF ERK MAPK in untreated piglets of both sexes, however significantly higher PRx values were seen in male versus female piglets. Following administration of U 0126, elevation of ERK MAPK levels was blocked in both sexes and PRx values were significantly improved in the male. A strong correlation was seen between the PRx and pial artery vasomotor activity. These data support previous observations that male piglets demonstrate reversible ERK MAPK-mediated impairment in autoregulation following FPI, which is reflected by the PRx. The strong correlation between the PRx and pial artery vasomotor activity supports the practice of continuously monitoring cerebrovascular autoregulation in patients using this index.

Keywords

Pressure reactivity index; ERK MAPK; Sex; Autoregulation; Pediatric traumatic brain injury

Introduction

Traumatic brain injury (TBI) is the leading cause of death and disability in children, with children ages 0–4 years and boys disproportionately represented [1–3]. Clinical data also suggest that boys suffer worse cognitive outcomes following TBI than girls after controlling for all other variables [4, 5]. These differences may in part be due to a greater impairment of cerebrovascular autoregulation in boys after injury. In the post-traumatic setting, cerebrovascular autoregulation, or the capacity of the cerebral blood vessels to maintain a constant flow despite changes in mean arterial pressure (MAP), is often disturbed [6–8]; this is compounded by the frequent occurrence of hypotension which has been associated with ischemic damage and poor outcome [9–13].

Pediatric TBI can be studied in piglets, which have a gyroencephalic brain, distribution of gray and white matter, cerebral growth pattern, and response to traumatic injury similar to humans [14–17]. Using a piglet model of pediatric TBI, previous studies have shown that while both sexes demonstrate increased cerebrovascular tone and impairment in cerebrovasodilation in response to hypotension after injury, this tendency is exacerbated in the male [18–20]. This is partially due to increased post-traumatic upregulation of the mitogen-activated protein kinase extracellular signal-related kinase (ERK MAPK) in the male, which has been associated with impairment of the appropriate vasodilatory response to hypotension [18, 19].

Clinically, cerebrovascular autoregulation may be monitored continuously by examining the relationship between slow waves in mean arterial blood pressure and intracranial pressure (ICP) using a value known as the pressure reactivity index or PRx [21]. The PRx is defined as the moving Pearson's correlation coefficient between MAP and ICP, and derives from the notion that in the normal brain, in low compliance states such as are frequently seen after TBI, increases in MAP will lead to constriction of the cerebral blood vessels in order to maintain constant flow. This results in a decrease in ICP due to a decrease in intracranial blood volume. PRx values range from –1 (perfectly intact autoregulation) to +1 (complete absence of autoregulation). Studies in both adults and children have demonstrated a strong relationship between PRx and outcome, with values greater than 0.2 associated with high morbidity and mortality [21–24]. The PRx has been shown to vary with cerebral perfusion pressure, making it a promising target for clinical therapeutics aimed at improving autoregulation and outcome. Because the PRx has been derived clinically, no comparison of PRx values with pial artery vasomotor response to hypotension, a more direct measurement of autoregulation, has been made. Similarly, the effects of sex and injury on pressure reactivity, in addition to what biological molecules may impact it, are unclear.

Given the observation that pial artery hypotensive dilation is impaired to a greater extent in male versus female piglets, and that this impairment in dilation is mediated by ERK MAPK, we hypothesized that the PRx would be elevated to a greater extent in male than female piglets after injury, and that this elevation would be ERK MAPK dependent. We further hypothesized that the PRx would vary with MAP after injury in our model, and would correlate well with pial artery dilatory response to hypotension.

Materials and Methods

Closed Cranial Window and Brain Injury Procedures

Newborn pigs (1–5 days old, weighing 1.0–1.6 kg) of both sexes were used. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Anesthesia was induced with isoflurane (1–2 MAC) and maintained with α -chloralose (30–50 mg/kg supplemented with 5 mg/kg/h IV). A catheter was inserted into the

femoral artery to monitor blood pressure and to sample blood gas tension 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 ventilated with room air. A heating pad was used to maintain the animals at 37–39 °C as monitored rectally.

A closed cranial window was inserted into the parietal skull of the anesthetized animals. This window consisted of three parts: a stainless steel ring, a circular glass cover slip, 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 millimolars): 3.0 KCl, 1.5 MgCl₂, 1.5 CaCl₂, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO₃. This artificial CSF was warmed to 37 °C and had the following chemistry: pH 7.33, PCO₂ 46 mmHg, and PO₂ 43 mmHg. Pial arterial vessel diameter was measured with a microscope, a camera, a video output screen, and a video microscaler.

FPI was induced using a device manufactured by the Medical College of Virginia using previously described methods [25]. A small craniotomy in the parietal skull contralateral to the cranial window was made, and a metal shaft was inserted into it on top of the intact dura. This shaft was connected to the transducer housing, which was in turn connected to a 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, and the other end had an acrylic plastic piston mounted on O-ring. 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 injury (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 injury 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

Small pial arteries (resting diameter 120–160 μm) were examined at the baseline MAP and during severe hypotension (40 % reduction) before and after injury. For sample collection, 300 μL of the total cranial window volume of 500 μL was collected by slowly infusing artificial CSF into one side of the window and allowing the CSF to drip freely into a collection tube on the opposite side.

Two experimental groups were studied: (1) FPI, vehicle post-treated, and (2) FPI, post-treated with ERK MAPK antagonist U 0126 (1 mg/kg IV). Pial artery diameter measurements and PRx calculations were done for 1-min intervals of four different blood pressure ranges both before and after injury: (1) 70–80 mmHg, (2) 60–70 mmHg, (3) 50–60 mmHg, and (4) 40–50 mmHg. These ranges were held constant by titration of additional blood withdrawal or blood for reinfusion. In some cases, boluses of saline and dilute norepinephrine (0.03–0.05 μg/kg) was necessary to maintain a MAP of 60–70 mmHg after injury. The vehicle for all agents was 0.9 % saline. Responses to papaverine (10⁻⁸ and 10⁻⁷ M), a vasoactive agent that elicits vasodilation unchanged by FPI and the ERK MAPK antagonist U 0126, were measured in order to indicate that the effects of brain injury, and pharmacologic probes on pial artery reactivity to hypotension are not an epiphenomenon [26, 27].

Calculation of the Pressure Reactivity Index

ICP was continuously monitored using an Integra Camino monitor (Integra Neurosciences, Plainsboro, NJ). MAP and ICP were continuously sampled and downloaded onto a personal computer at a rate of 60 Hz. A program was written in Labview to store these values, and calculate average MAP and ICP at each 1-s interval. The PRx was then calculated every minute, using a 5-min moving window, as the Pearson's correlation coefficient between ICP and MAP as previously described [26]. PRx determination was done using Excel. The average PRx for every 10-min blood pressure interval both before and after injury was determined for each animal.

Enzyme-Linked Immunosorbent Assay

Commercially available enzyme-linked immunosorbent assay kits were used to quantify CSF ERK MAPK (Assay Designs, Ann Arbor, MI) concentrations. Phosphorylated ERK MAPK enzyme values were normalized to the total ERK MAPK form value, and expressed as a percentage of the control condition.

Statistical Analyses

Pial artery diameter, CSF ERK MAPK, and PRx in the 60–70-mmHg range were analyzed using ANOVA for repeated measures. The average PRx for each blood pressure range was also determined for each group of animals, and analyzed using ANOVA for repeated measures. An α level of $p < 0.05$ was considered significant in all statistical tests. The percent change in both cerebral arterioles and small arteries was compared to the average PRx in the 60–70-mmHg range for all animals both before and after injury using the Spearman rank order coefficient. Values are presented as mean $\pm$ SEM of the absolute value.

Results

FPI Leads to an Upregulation of ERK MAPK in Males and Females

Phosphorylated ERK MAPK was significantly elevated following fluid percussion injury in both male and female piglets, but was elevated to a greater extent in males. Post-treatment with U 0126 blocked the upregulation of ERK MAPK in both sexes (Fig. 1).

Pressure Reactivity is Impaired in Males More than Females and Related to ERK MAPK

Before injury, PRx values reflecting intact autoregulation with no significant sex-based differences were seen across all MAP ranges in both treated and untreated animals (Fig. 2). However, after injury, PRx values in untreated males were significantly greater than those of untreated females in all MAP ranges. After treatment with ERK MAPK inhibitor U 0126, the PRx values of male piglets were significantly lower than those of untreated males, and comparable to those of both treated and untreated females. No significant relationship was seen between the PRx and mean arterial blood pressure range before or after injury in either treated or untreated males or females (Fig. 2).

Pressure Reactivity Correlates with Pial Artery Dilation to Hypotension

Impaired pial artery dilation was seen in response to hypotension after injury in both the female and male piglet groups, though impairment was more profound in the males. The administration of ERK MAPK antagonist U 0126 restored hypotensive vasodilation more robustly in the male than female piglets (Fig. 3). Using data from untreated animals of both sexes before and after injury, the percent change in small pial artery diameter in response to severe hypotension (40 % decrease in blood pressure) correlated well with the PRx at a neutral blood pressure range (50–60 mmHg; $r = -0.8714$, $p < 0.01$; Fig. 4). Vasodilation in

response to papaverine was not affected by sex, injury, or ERK MAPK expression (data not shown).

Blood Chemistry

Temperature and pCO₂ data are provided in Table 1. No statistical differences in blood chemistry were seen between animals before or after injury or following the administration of U 0126 or vehicle. The amplitude of the pressure pulse was equivalent in male and female vehicle and drug-treated animals.

Discussion

The results of this study demonstrate that pressure reactivity in newborn piglets varies as a function of sex, injury, and ERK MAPK, with male piglets demonstrating ERK MAPK-dependent impairment in pressure reactivity after injury. No significant relationship between MAP and the PRx was seen. Lastly, we found a strong correlation between pressure reactivity and pial artery dilation in response to hypotension, providing additional support for the use of this measure to continuously evaluate autoregulation in children at the bedside.

Our findings are consistent with previous observations that FPI leads to the upregulation of ERK MAPK in a sex-dependent manner, and that this upregulation is associated with disturbed autoregulation [18–20]. We reported impairment in the autoregulatory index following FPI in both male and female piglets, which was reversed with the administration of adrenomedullin, a neuropeptide which was observed to limit upregulation of ERK MAPK after FPI [18]. This study extends these results by demonstrating that a blockade of ERK MAPK similarly improves pressure reactivity in the male piglet. ERK MAPK may therefore represent an important therapeutic target, particularly for boys with traumatic brain injury.

A key finding of this study is that the PRx, a metric that has been derived clinically and relies on several physiologic assumptions, corresponds well with pial artery responsiveness to hypotension, a direct way of assessing autoregulation [21]. The PRx was first described by Czosnyka et al. [28] in 1996 as a way of continuously assessing cerebral pressure reactivity, a physiologic property similar but not identical to autoregulation, by examining the relationship between slow, spontaneous fluctuations in arterial blood pressure and intracranial pressure. PRx values in adult patients greater than +0.2 have been associated with a high rate of unfavorable outcome, while those below 0.05 have been associated with good outcome [22–24]. Brady et al. examined the PRx in a small group of pediatric patients with severe traumatic brain injury, and similarly demonstrated higher PRx values in patients with poor outcome versus those with a good outcome [29]. Because the PRx varies with CPP, autoregulation can theoretically be improved at the bedside, and there is some evidence that maintenance of an optimal CPP results in better outcomes [22, 24]. Although there is much excitement surrounding use of the PRx, a major criticism is that its relationship to autoregulation is unclear [21]. The results of this study provide direct support for a relationship between calculated PRx values and vasomotor autoregulatory responses.

An assumption about the PRx is that intracranial compliance must be low for the value to be valid [21]. However, Brady et al. demonstrated that comparable PRx values were calculated in piglets with ICPs artificially maintained at 20 mmHg by infusing artificial CSF into the CSF space, and when naïve ICPs were maintained [30]. Similar to our study, no relationship was seen between the PRx and MAP in uninjured piglets above the lower limit of autoregulation (about 30 mmHg).

There were several limitations of this study. First, we chose to examine the relationship between the PRx and MAP instead of CPP to allow for easier comparison with previous

studies, and because ICP monitoring is uncommon in children, particularly those less than 2 years of age [31]. In most cases, the CPP was 2–8 mmHg below MAP and greater than the lower limit of autoregulation in piglets, except following injury in some untreated piglets while a MAP range of 40–50 was induced. Second, the 10-min sampling period we used to calculate the PRx was significantly shorter than the minimum of 2 h that is suggested when determining this value in patients [20]. Such long periods of time were not feasible in this study, however our results followed the expected overall trends. Nonetheless, these small sampling periods likely contributed to the variability in our PRx measurements and may explain why no relationship was seen between PRx and MAP after injury in our piglets. Third, some piglets required boluses of dilute norepinephrine following injury to maintain a MAP of 60–70 mmHg following injury. We have previously shown that a continuous infusion of phenylephrine exacerbates sex-related disparities in autoregulation when administered following fluid percussion injury. Because only small amounts were used for short periods of time in both sexes, and ERK MAPK assays demonstrating greater post-traumatic upregulation in the male versus female piglet were collected before the administration of any drug, we do not believe this significantly affected our results. Future studies will be required to determine whether other vasoactive molecules such as endothelin-1, which has been associated with impaired autoregulation following FPI, also have a sex-dependent effect on the PRx, and whether a relationship between MAP and PRx might be demonstrated with longer monitoring periods [32, 33]. Lastly, this study did not utilize a control group, however we have previously published that U 0126 has no effect on pial artery diameter and CBF in sham control (non-FPI) animals [27].

Conclusion

In conclusion, the results of this study confirm previous findings that male piglets demonstrate a greater degree of ERK MAPK-related impairment in autoregulation following FPI than females, and demonstrate similar trends using a clinically relevant method for continuously monitoring pressure reactivity, the PRx. This provides more evidence that ERK MAPK may be a potential therapeutic target for boys with TBI. A significant relationship was also demonstrated between the PRx and pial artery dilatory response to hypotension, supporting the use of this measurement for the real-time evaluation of autoregulation patients, particularly children.

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Fig. 1. Phosphorylation of ERK MAPK as a function of injury, sex, and ERK MAPK inhibition with U 0126. (* $p < 0.05$ compared to sham; # $p < 0.05$ compared to U 0126-treated animal; *ERK* extracellular signal-related kinase, *MAPK* mitogen-activated protein kinase, *ANOVA* analysis of variance, *ELISA* enzyme-linked immunosorbent assay)

Fig. 2.

Relationship between MAP, FPI, sex, and ERK MAPK and the PRx. The mean $PRx \pm SEM$ is presented at each MAP range for both treated and untreated animals of both sexes before and after injury. Untreated males and females are shown **a** pre- and **b** post-injury. Males and females treated with U 0126 (10^{-6} M) 30 min after injury are shown **c** before and **d** after injury (* $p < 0.05$ compared to pre-injury untreated animals, post-injury females, and post-injury treated males and females)

Fig. 3.

The change in pial artery diameter in response to hypotension is shown as a percentage of the resting diameter for each group both before and after injury (* $p < 0.05$ compared to the corresponding non-motensive pial artery diameter, # $p < 0.05$ compared to corresponding pre-injury value, + $p < 0.05$ compared to corresponding female value, and & $p < 0.05$ compared to untreated male value)

Fig. 4.

PRx is shown plotted against the percent change in pial artery diameter in response to severe hypotension. A high correlation was seen between these two values in untreated animals of both sexes before and after injury ($r=-0.8714$, $p<0.01$). Data analyzed using Spearman rank order coefficient

Table 1Temperature and pCO₂ data

	Temperature (mean±SEM)	pCO ₂ (mean±SEM)
Untreated males	37±0.5	34.1±1.1
Untreated females	37±0.7	34.7±1.3
Treated males	38±0.5	35.1±0.8
Treated females	38±0.3	37.3±1.0