Heat shock protein modulation of $K_{\text{ATP}}$ and $K_{\text{Ca}}$ channel cerebrovasodilation after brain injury

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Armstead, William M., and James G. Hecker. Heat shock protein modulation of $K_{\text{ATP}}$ and $K_{\text{Ca}}$ channel cerebrovasodilation after brain injury. Am J Physiol Heart Circ Physiol 289: H1184–H1190, 2005. First published May 20, 2005; doi:10.1152/ajpheart.00276.2005.—Fluid percussion brain injury (FPI) impairs pial artery dilation to activators of the ATP-sensitive ($K_{\text{ATP}}$) and calcium-activated ($K_{\text{Ca}}$) $K^+$ channels. This study investigated the role of heat shock protein (HSP) in the modulation of $K^+$ channel-induced pial artery dilation after FPI in newborn pigs equipped with a closed cranial window. Under nonbrain injury conditions, topical coadministration of exogenous HSP-27 (1 $\mu$g/ml) potentiated dilation to cromakalim, CGRP, and NS-1619. FPI increased the cerebrospinal fluid (CSF) concentration of HSP-27 from 0.051 ± 0.012 to 0.113 ± 0.035 ng/ml but decreased the CSF concentration of HSP-70 from 50.42 ± 8.96 to 30.9 ± 9.9 ng/ml at 1 h posts insult. Pretreatment with topical exogenous HSP-70 (1 $\mu$g/ml) before FPI fully blocked injury-induced impairment of cromakalim and CGRP dilation and partially blocked injury-induced impairment of dilation to NS-1619. These data indicate that HSP-27 and HSP-70 contribute to modulation of $K^+$ channel-induced pial artery dilation. These data suggest that HSP-70 is an endogenous protectant of which its actions may be unmasked and/or potentiated with exogenous administration before brain injury.

ATP-sensitive potassium channel; calcium-activated potassium channel; cerebral circulation; newborn; ion channels

RELAXATION OF BLOOD VESSELS can be mediated by several mechanisms, including guanosine 3',5'-cyclic monophosphate cGMP, cAMP, and $K^+$ channels (14). Membrane potential of vascular muscle is a major determinant of vascular tone, and activity of $K^+$ channels is a major regulator of membrane potential (30). 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 (30). There are at least four types of $K^+$ channels: ATP-sensitive ($K_{\text{ATP}}$), calcium-activated ($K_{\text{Ca}}$), the delayed rectifier, and the inward rectifier. Direct measurements of membrane potential and $K^+$ current in vitro indicate that several different types of $K^+$ channels are present in cerebral vessels. A number of pharmacological studies have provided functional evidence that $K^+$ channels, especially $K_{\text{ATP}}$ and $K_{\text{Ca}}$, regulate the tone of cerebral vessels in vitro and in vivo (14). Because selective agonists and antagonists are available for study of $K_{\text{ATP}}$ and $K_{\text{Ca}}$ channels, substantial evidence has been accumulated to indicate that these channels contribute to both the physiological and pathophysiological regulation of the cerebral circulation (14). In the piglet cerebral circulation, cross-selectivity experiments have shown that cromakalim and CGRP are selective synthetic and endogenous activators of the $K_{\text{ATP}}$ channel, respectively, whereas NS-1619 is a synthetic activator of the $K_{\text{Ca}}$ channel (1–3). Pial arteries are innervated by CGRP-containing nerve fibers (13), whereas CGRP produces hyperpolarization of cerebral vascular muscle in vitro (41).

Traumatic injury is the leading cause of death for infants and children, and mortality is greatly increased in the presence of concussive head injury (40). Whereas the effects of brain injury have been investigated extensively in the adult, less is known about brain injury in the newborn/infant. Cerebral blood flow decreases and pial arteries contract more in newborn (1–5 day old) versus juvenile (3–4 wk old) pigs after fluid percussion brain injury (FPI), a model of concussive head injury, supporting the idea that the newborn has greater cerebral hemodynamic sensitivity to brain injury (5). With the use of pial artery dilation as an index of channel activity, it was observed that $K^+$ channel impairment contributes substantially to age-dependent altered cerebral hemodynamics after FPI (5, 6).

Numerous insults, such as elevated temperature and ischemia, lead to the expression of heat shock proteins (HSPs). Expression of HSPs results in tolerance, an inducible form of protection (19). Although mammalian cells are rarely exposed to heat shock, this model system is useful for the study of other conditions in which damaged proteins accumulate, such as traumatic brain injury. There are several major types of HSPs, including HSP-20, -27, -70, and -90 (8). HSP-70 is expressed after human traumatic brain injury (32), FPI in the adult rat (45), closed head injury using a weight-drop method in the adult mouse (43), and spinal cord injury in the adult rat (44). Preadministration of a $K_{\text{ATP}}$ agonist prevented global ischemia-induced HSP-70 expression in the rat hippocampus (16). Alternatively, HSP-70 has been observed to activate the $K_{\text{Ca}}$ channel in human promonocyte U-937 cells (29). Therefore, the relationship between HSP and $K^+$ channels may be reciprocal; however, this area is poorly understood. Furthermore, some HSPs may produce vasoconstriction (e.g., HSP-27), whereas others (e.g., HSP-20) elicit vasodilation (10, 38). Thus the functional significance of an HSP and $K^+$ channel relationship in the context of traumatic brain injury is provocative but uncertain.

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This study was designed to investigate the role of HSP in the modulation of K⁺ channel-induced pial artery dilation after FPI in the newborn pig.

MATERIALS AND METHODS

Newborn pigs (1–5 day old, 1.2–1.8 kg) of either sex were used in these experiments. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Animals were sedated with isoflurane (1–2 minimum alveolar concentration). Anesthesia was maintained with α-chloralose (30–50 mg/kg supplemented with 5 mg·kg⁻¹·h⁻¹ 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, with temperature monitored rectally.

A cranial window was placed 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 cerebrospinal fluid (CSF), of the following composition (in mM): 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, which was similar to that of endogenous CSF: pH 7.33, PCO₂ 46 mmHg, and PO₂ 43 mmHg. 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 microcalser.

Methods for brain FPI have been described previously (48). 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. A metal shaft was then 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 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. Two types of pial 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. For sample collection, 300 μl of the total cranial window volume of 500 μl were collected by slowly infusing CSF into one side of the window and allowing the CSF to drip freely into a collection tube on the opposite side.

Five types of experiments were performed (all n = 6) as follows: 1) sham control, 2) HSP-27 treated, 3) HSP-70 treated, 4) FPI, and 5) FPI and HSP-70 treated. In sham control experiments, responses to cromakalim, CGRP, and NS-1619 (10⁻⁸ and 10⁻⁶ M) were obtained initially and then again 60 min later to determine whether responses were reproducible over time. In the second and third group of experiments, responses to the topical administration of K⁺ channel agonists were obtained initially and then again 60 min after the topical administration of either exogenous HSP-27 or HSP-70 (1 μg/ml; Stressgcn). In the fourth group, responses to the K⁺ channel agonists were obtained initially and then again 60 min after FPI. Finally, in the fifth group, responses to the K⁺ channel agonists were obtained initially, exogenous HSP-70 was topically applied 30 min before FPI, and responses were then obtained again 60 min after FPI. In the second, third, and fifth groups, the respective HSP was coadministered with each of the agonists during the generation of the dose-response curves to maintain continued exposure to the respective HSP. Because baseline artery diameter changed as a result of FPI, data were calculated as the percentage change from baseline to normalize such differences.

ELISA analysis of CSF for HSP. Commercially available ELISA kits (Stressgcn) were used to quantify CSF HSP-27 and HSP-70 concentration. In the first step, the CSF is introduced into a microwell coated with a highly purified monoclonal antibody specific for the HSP. HSP-27 or HSP-70, if present in the sample, will bind to the solid phase antibody. Subsequently, a second noncompeting anti-HSP antibody labeled with horseradish peroxidase is added. With a positive reaction, this labeled antibody becomes bound to any solid phase antibody–HSP complex previously formed. Incubation with enzyme substrate produces a blue color. The amount of color developed is directly proportional to the concentration of HSP in the tested sample.

Statistical analysis. Pial artery diameter, CSF HSP-27 and HSP-70 concentration, and systemic arterial pressure values were analyzed using ANOVA for repeated measures. If the value was significant, the data were then analyzed by Fishers protected least significant difference test. An α level of P < 0.05 was considered significant in all statistical tests. Values are means ± SE of the absolute value or as percentage changes from control values.

RESULTS

FPI alters CSF HSP-27 and HSP-70 concentration. Cortical paracrine HSP CSF samples were obtained before FPI and at 1 and 4 h after FPI (Fig. 1). CSF HSP-27 concentration was elevated progressively after FPI, whereas CSF HSP-70 concentration was progressively decreased postinsult (Fig. 1).

HSP-27 blunts whereas HSP-70 potentiates K⁺ channel agonist-induced pial artery dilation in noninjured newborn pigs. Cromakalim, CGRP, and NS-1619 (10⁻⁸ and 10⁻⁶ M) elicited reproducible pial small artery (120–160 μm) and arteriole (50–70 μm) dilation. Coadministration of exogenous HSP-27 (1 μg/ml) with cromakalim, CGRP, and NS-1619 blunted artery dilation to these K⁺ channel agonists (Figs. 2 and 3). On a percentage basis, pial artery dilation was blunted by 67 ± 2% and 58 ± 4% for cromakalim (10⁻⁸ and 10⁻⁶ M), and a similar percentage of inhibitions was observed for the effects of HSP-27 on CGRP and NS-1619 dilation. HSP-27 by itself did not have any significant effect on pial artery diameter (161 ± 9 vs. 169 ± 6 μm, respectively; n = 6). In contrast, coadministration of exogenous HSP-70 (1 μg/ml) potentiated cromakalim-, CGRP-, and NS-1619-induced pial artery dilation (Figs. 2 and 3). On a percentage basis, pial artery dilation was potentiated by 52 ± 8 and 49 ± 8% for cromakalim (10⁻⁸ and 10⁻⁶ M), and similar percentage changes were observed for CGRP and NS-1619. However, dilation to the nonselective vasodilator papaverine was unchanged by coadministration of either HSP-27 or HSP-70 (Fig. 4). HSP-70 by itself did not have any significant effect on pial artery diameter (142 ± 6 vs.
136 ± 5 μm, respectively, n = 6). Similar observations were made in pial arterioles.

HSP-70 pretreatment partially restores impaired K⁺ channel agonist-induced pial artery dilation in newborn pigs after brain injury. Cromakalim-, CGRP-, and NS-1619-induced pial artery dilations were blunted within 60 min after FPI (Figs. 5 and 6). However, pretreatment with exogenous HSP-70 (1 μg/ml) 30 min before FPI along with continued coadministration of this agent with the K⁺ channel agonists postinjury fully restored decremented pial artery dilation to cromakalim and CGRP (Fig. 5). Impaired vasodilator responses to NS-1619 postinjury were partially restored by pretreatment with HSP-70 (Fig. 6).

Blood chemistry, systemic arterial blood pressure, baseline pial artery diameter, and intensity of injury. Blood chemistry and systemic arterial blood pressure values were obtained at the beginning and end of all experiments. These values were 7.48 ± 0.03, 36 ± 4, 93 ± 9, and 65 ± 5 mmHg vs. 7.46 ± 0.04, 34 ± 7, 90 ± 10, and 52 ± 5 mmHg for pHe, PCO₂, and PO₂, respectively, before and after injury. There were no statistical differences in the blood chemistry parameters among the sham-, FPI-, and HSP-treated animals. The mean systemic arterial blood pressure was decreased by FPI but was constant during pial artery dose-response curve generation for the effects of topical K⁺ channel agonists. The baseline pial small artery diameter was decreased from 153 ± 9 to 120 ± 6 μm within 60 min of FPI; however, HSP-70 administration blocked such change (155 ± 9 and 151 ± 8 μm). Neither HSP-27 nor HSP-70 by themselves had any significant effect on pial artery diameter in sham animals. The amplitude of the pressure pulse, used as an index of injury intensity, was equivalent in all experimental groups of pigs (1.9 ± 0.1 atm).
DISCUSSION

The results of the present study show that cortical periarachnoid CSF HSP-27 concentration was elevated within 1 h of FPI. CSF HSP-27 concentration continued to increase for 4 h after FPI. In contrast, CSF HSP-70 concentration progressively decreased over the same time period after brain injury. On a relative basis, there was more HSP-70 in CSF under resting physiological (before brain injury) conditions when compared with HSP-27. However, the concentration present at the receptor level is unknown but is presumably somewhat greater. These data indicate that HSP-27 and HSP-70 are detectable in CSF and that brain injury modulates the relative amounts of these HSPs. It was somewhat surprising that the CSF HSP-70 concentration progressively decreased with time after FPI. The reasons for this occurrence are uncertain but could relate to the sampling site (the cranial window was located contralaterally to the injury site), metabolism of the HSPs, and the age and/or species of animal investigated. The experimental design of the present study did not allow for the identification of the cellular site of origin for the HSPs detected in CSF. Potential cellular sites of origin include neurons, glia, vascular smooth muscle, and endothelial cells. It is speculated that the HSP release profile may vary as a function of region and cell type as well as time postinsult.

Other results of this study show that pial artery dilation induced by activators of the \( K_{ATP} \) and \( K_{Ca} \) channel was blunted by the coadministration of exogenous HSP-27 in nonbrain-injured animals. Conversely, coadministration of exogenous HSP-70 potentiated the vasodilation observed in response to the \( K^+ \) channel agonists. In these studies, vasodilation was used as an index of \( K_{ATP} \) channel function. These data indicate that HSP-27 and HSP-70 modulate \( K^+ \) channel function. This modulation appears selective in that responses to papaverine were unchanged. The concentration of HSP-27 and HSP-70 used in these experiments was somewhat arbitrarily chosen, though several literature examples describe similar concentrations of HSP-27 and HSP-20 used to modulate vascular tone or dilator responses to sodium nitroprusside (15, 38). Little is known regarding quantitative data for HSP concentration in the brain. Whereas the concentrations of HSPs at the receptor level are uncertain, the present data suggest that concentrations of HSP-27 and HSP-70 that might be considered pathophysiological or pharmacological modulate \( K^+ \) channel function.

Fig. 4. Influence of HSP-27 (1 µg/ml) and HSP-70 (1 µg/ml) coadministration with papaverine (10\(^{-8}\) and 10\(^{-6}\) M) on pial artery diameter; \( n = 6 \).

Fig. 5. Influence of cromakalim and CGRP (10\(^{-8}\) and 10\(^{-6}\) M) on pial artery diameter before (control), after FPI, and after FPI in HSP-70 (1 µg/ml)-pretreated animals; \( n = 6 \). *\( P < 0.05 \) vs. corresponding control; +\( P < 0.05 \) vs. corresponding nonpretreated value.

Fig. 6. Influence of NS-1619 (10\(^{-8}\) and 10\(^{-6}\) M) on pial artery diameter before (control), after FPI, and after FPI in HSP-70 (1 µg/ml)-pretreated animals; \( n = 6 \). *\( P < 0.05 \) vs. corresponding control; +\( P < 0.05 \) vs. corresponding nonpretreated value.
cause observations regarding the modulation of K⁺ channel-induced vasodilation were similar in pial small arteries and arterioles, these data suggest that there are minimal regional vascular differences in such modulation of vascular activity.

Additional results of the present study show that cromakalim-, CGRP-, and NS-1619-induced pial artery dilations were blunted within 60 min after FPI, consistent with previous observations (3–6). However, pretreatment with HSP-70, in the same concentration used in the noninjury studies (see HSP-27 blunts whereas HSP-70 potentiates K⁺ channel agonist-induced pial artery dilation in noninjured newborn pigs), 30 min before FPI along with continued coadministration of this agent with the K⁺ channel agonists postinjury fully restored decremented pial artery dilation to cromakalim and CGRP. Impaired vasodilator responses to NS-1619 postinjury were partially restored by pretreatment with HSP-70. Previous studies have shown that pial dilator responses to papaverine were unchanged after FPI in the piglet (4), thereby providing symmetry to the above studies wherein responses to papaverine were unchanged by HSP-70 under nonbrain injury conditions. Therefore, these data suggest that the protection observed with pretreatment by HSP-70 before injury should be selective for K⁺ channel agonists. Previous studies (4) have shown that dilation to the K⁺ channel agonists was blunted at 4 h after FPI to an extent nonsignificantly different from that observed at 1 h postinsult. These data show symmetry in the time course between the decrease in CSF HSP-70 and impaired dilation to K⁺ channel agonists after brain injury. These data suggest that responses to K⁺ channel agonists may be blunted after FPI because the concentration of this endogenous protectant is decreased postinsult. Therefore, these data suggest that HSP-70 is an endogenous protectant of which its actions may be unmasked and/or potentiated with therapeutic interventions such as gene overexpression. Interesting, though, is the observation that the dilation to the Kₐtg channel agonist is more robustly protected by HSP-70 postinsult when compared with that observed with the Kₐ channel agonist. These data suggest that other HSPs may contribute to the modulation of Kₐ channel-mediated vasodilation to a greater extent and thereby indicate a different therapeutic HSP isoform intervention for this K⁺ channel subtype. These data, however, are not necessarily inconsistent with the recently published clinical data showing that CSF HSP-70 concentration was elevated more in victims of child abuse than in victims of accidental brain injury (25). In the latter study, because it was clinical, CSF HSP levels before brain injury were not available. Additionally, only a single time point sample was available in most cases. Therefore, the direction of change in concentration as a function of time after brain injury was unknown, only that the relative levels were greater in abuse versus accidental brain injury. Thus, though levels of the putatively protective HSP-70 may have been greater in cases of abuse, such levels may still have been suboptimal and amenable for therapeutic intervention, which could further elevate HSP-70 concentration.

HSPs are proteins that are induced by a wide variety of stresses, including oxidative and ischemic injury (12, 46). HSPs participate as molecular chaperones in protein translocation and folding (7, 42) as well as contribute to cellular repair processes by refolding denatured proteins (33). HSPs also assist in monitoring and controlling other specific protein–protein interactions, such as assembly and disassembly of protein complexes and presentation of substrates for degradation (31). HSPs are generally divided into two groupings: the high-molecular-weight HSPs, including HSP-90 and HSP-70, and the low-molecular-weight HSPs, including HSP-27 and HSP-20 (8). Small HSPs are involved in smooth muscle contraction (HSP-27) and relaxation (HSP-20) (26). HSP-27 may modulate contractility through effects on the actin cytoskeleton because increased HSP-27 phosphorylation affects actin microfilament dynamics (26), whereas changes in the association of HSP-27 with actin occur during sustained smooth muscle contraction (20, 47). Additionally, it has been observed that phosphorylation of HSP-20 is important for cyclic nucleotide-dependent smooth muscle relaxation (38, 39, 49) and that phosphorylated HSP-27 inhibits this mechanism (15). The latter suggests that a balance between HSP-27 and HSP-20 may regulate the level of contraction (31). Impaired relaxation of cerebral vessels in a rat subarachnoid hemorrhage model was associated with increases in the amount of phosphorylated HSP-27 and decreases in the expression and phosphorylation of HSP-20 (28). However, the role of large HSPs (e.g., HSP-70 and HSP-90) in the modulation of vascular tone is unknown.

HSP-70 appears to be involved in protection from cerebral ischemic death. During sham conditions, the amount of HSP-70 is low, whereas its expression is elevated after ischemia (12, 46). In speculation that such increases were beneficial, studies have been designed to augment the levels of HSP-70 under ischemic injury conditions. In these studies, augmented levels of HSP-70 achieved either by transgenic overexpression (34, 36) or induced overexpression with either the herpes or adenoviral vector reduced injury in models of both global and focal ischemia (21, 22, 50). In fact, delayed administration of the HSP-70 vector up to 2 h postinsult was still protective (18). However, such results were not uniform (27). Explanations in those cases included an insult that was too severe, not severe enough, or expression of HSP-70 that was too low (11, 27).

A relationship between K⁺ channels and HSPs has been noted previously (17, 29), particularly with reference to the above (22) described role for HSP in ischemic protection. For example, it has been proposed that HSP-70 activates the Kₐ channel to elicit preconditioning after ischemia (11, 17, 24), though this was not necessarily observed in all cases (9, 37). Similarly, HSP-27 may be involved in cortical spreading depression (K⁺ mediated)-induced ischemic tolerance (35). Regarding studies that do not involve ischemic protection, HSP-70 has been observed to activate the Kₐ channel in human promonocyte U-937 cells (29) and to augment KCl contraction in the isolated rat aorta (23). However, nothing is known regarding the relationship between HSPs and dilation in response to K⁺ channel agonists.

Previous studies (5, 6) have observed that Kₐ and Kₐ-dependent dilator stimulus (5), a Kₐ and Kₐ-dependent dilator stimulus (5). Whereas the role of endogenous substance release (e.g., endothelin, vasopressin, the opioid nociceptin/orphanin FQ) after injury in the impairment of K⁺ channel-mediated vasodilation has been rather well explored (5, 6), the converse has not previously been
investigated. In particular, it has been heretofore uncertain if a substance might be released after FPI which could protect vascular responsiveness. Data in the present study suggest that HSP-70 may be that endogenous protectant. Although the CSF HSP-70 concentration was only observed to decrease postinjury, clinical data (25) suggest that the time frame for observation of such an increased concentration may not have been optimally chosen in the present study. The decrease in CSF HSP concentration after FPI is not necessarily inconsistent with the notion that K⁺ channel function impairment relates to suboptimal concentrations of endogenous protectant(s) in the brain. Exogenous administration of the putative protectant HSP-70 robustly fully restored the previously impaired vasodilation to KATP channel agonists after FPI. The identification of an endogenous protectant could allow for the development of new avenues of therapy such as gene overexpression. Nonetheless, caution is urged because in the present study animals were pretreated with HSP-70 before injury. The merit of an endogenous protectant could allow for the development and the University of Pennsylvania Research Foundation.

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