Apolipoprotein-E Controls Adenosine Triphosphate-Binding Cassette Transporters ABCB1 and ABCC1 on Cerebral Microvessels After Methamphetamine Intoxication

Ayman ElAli, PhD; Andrés Urrutia, MSc; Ana Rubio-Araiz, PhD; Macarena Hernandez-Jimenez, PhD; Maria I. Colado, PhD; Thorsten R. Doeppner, MD, MSc; Dirk M. Hermann, MD

***Background and Purpose*—**Methamphetamine is a powerful addictive, which has been associated with ischemic stroke and brain hemorrhage in humans. Whether and how methamphetamine influences the expression of tight junctions and adenosine triphosphate-binding cassette transporters, which have previously been shown to be regulated by apolipoprotein-E (ApoE) under conditions of brain ischemia, was unknown.

***Methods*—**C57BL/6J mice received intraperitoneal injections of methamphetamine (3 times 4 mg/kg separated by 3 hours) either alone or in combination with the ApoE receptor-2 inhibitor receptor-associated protein (40 μg/kg) or the inducible nitric oxide synthase inhibitor 1400W (5 mg/kg). Animals were euthanized 3 or 24 hours after methamphetamine exposure. Tissue responses were evaluated with Western blots, immunoprecipitation, and immunohistochemistry using total brain and cerebral microvessel extracts.

***Results*—**Methamphetamine induced a transient activation of stress kinases c-Jun N-terminal kinase 1/2 and p38 in the brain parenchyma and increased intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression on cerebral microvessels without inducing loss of tight junction proteins and without inducing IgG extravasation. Methamphetamine transiently increased the expression of the luminal adenosine triphosphate-binding cassette transporter ABCB1 on cerebral microvessels and reduced the expression of the abluminal transporter ABCC1. Elevated expression of ApoE was noted in the brain parenchyma by methamphetamine, activating ApoE receptor-2 on brain capillaries, deactivating c-Jun N-terminal kinase 1/2 and c-Jun, and regulating ABCB1 and ABCC1 expression. Indeed, ApoE receptor-2 and inducible nitric oxide synthase inhibition prevented the ABCB1 and ABCC1 expression changes.

***Conclusions*—**Acute exposure to methamphetamine at doses comparable to those consumed in drug addiction does not induce tight junction breakdown but differentially regulates adenosine triphosphate-binding cassette transporters through the ApoE/ApoE receptor-2/c-Jun N-terminal kinase 1/2 pathway. **(*Stroke*. 2012;43:1647-1653.)**

Key Words: apolipoprotein-E receptor-2 ■ blood–brain barrier ■ c-Jun N-terminal kinase 1/2 ■ drug abuse ■ endothelium ■ multidrug resistance ■ neuropathology ■ signal transduction ■ tight junction

Downloaded from http://ahajournals.org by on July 6, 2023

Methamphetamine (also called crystal meth or meth) is a psychostimulant and addictive that causes severe health problems mainly due to neurotoxicity, cardiac arrhythmia, and hyperthermia.[1,2] In the brain, meth overactivates the dopaminergic system, disrupting synaptic integrity[3] and inducing neuronal injury through oxidative stress.[4,5] Meth may also induce neuroinflammation[6,7] and increase blood–brain barrier (BBB) permeability.[8,9] Possibly related to such vascular actions, meth abuse has been shown to be associated with ischemic stroke and intracerebral and subarachnoid hemorrhage in humans.[10]

The BBB is formed by endothelial cell–cell contacts, the tight junctions,[11] which are complemented by transporters on the luminal and abluminal endothelial membranes belonging to the adenosine triphosphate-binding cassette (ABC) transporter and solute carrier families.[12,13] Whereas tight junctions are responsible for maintaining paracellular BBB tightness, ABC transporters control brain homeostasis and protect the brain from environmental molecules by eliminating them across the BBB.[12,13] In vitro, meth exposure has previously been shown to deregulate tight junction proteins[14–16] and impair the expression of a solute carrier, the glucose trans-

Received December 21, 2011; final revision received January 19, 2012; accepted January 30, 2012.

From the Department of Neurology (A.EA., T.R.D., D.M.H.), University Hospital Essen, Essen, Germany; and the Departamento de Farmacologia (A.U., A.R.-A., M.H.-J., M.I.C.), Facultad de Medicina, Universidad Complutense, Madrid, Spain.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.111.648923/-/DC1.

Correspondence to Dirk M. Hermann, MD, Department of Neurology, University Hospital Essen, Hufelandstr 55, D-45122 Essen, Germany. E-mail dirk.hermann@uk-ussen.de

© 2012 American Heart Association, Inc.

***Stroke* is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.111.648923**

porter protein-1.[16] In vivo, the effect of meth on tight junction integrity and ABC transporter expression was unknown. Meth has previously been proposed to act as an ABC transporter substrate.[17]

ABC transporters are actively regulated in the brain under various pathophysiological conditions.[12,13] In focal cerebral ischemia, our group has previously shown that the transporter ABCB1 (previously: multidrug resistance transporter-1), which is localized on the luminal membrane of brain endothelial cells and extrudes a broad range of substrates—among them also pharmacological drugs—from the vessel into the blood, is upregulated,[18] whereas the transporter ABCC1 (previously: multidrug resistance-associated protein-1), which is expressed on the abluminal endothelial membrane and carries its substrates—among them various cell metabolites—in the opposite direction from the vessel into the brain, is downregulated on cerebral microvessels.[19] ABCB1 and ABCC1 expression were controlled by apolipoprotein-E (ApoE), which deactivates c-Jun N-terminal kinase-1/2 (JNK1/2) and its downstream kinase c-Jun through its receptor ApoER2 (also: low-density lipoprotein receptor-related protein-8), thereby modulating ABC transporter transcription.[20]

Based on these earlier results, we were interested in how an acute meth exposure at doses comparable to those used in drug addiction influences the integrity of tight junctions and ABC transporter expression on brain capillary cells. A deregulation of tight junctions might be relevant with respect to stroke pathogenesis, because it may reveal a mechanism by which meth may provoke cerebral thromboembolism and brain hemorrhage.[10] The regulation of ABC transporters, on the other hand, might unravel a strategy through which the brain protects itself from intoxication.[21]

Materials and Methods

Animal Groups and Methamphetamine Exposure

All animal experiments were done according to the National Institutes of Health guidelines for the care and use of laboratory animals based on guidelines of the local Animal Welfare Committee of the Universidad Complutense de Madrid. Adult male C57BL/6j mice (Harlan, Barcelona, Spain) weighing 25 to 30 g were kept under conditions of constant ambient temperature (21±2°C) at a 12-hour light/dark cycle (lights on at 8:00 AM) with free access to food and water. Meth (4 mg/kg, dissolved in 0.9% NaCl; Sigma, Madrid, Spain) or vehicle (0.9% NaCl) was intraperitoneally administered 3 times at 3-hour intervals. A first set of mice was divided into 3 groups receiving (1) vehicle treatment followed by 3 hours survival starting with the last injection (n=7 animals); (2) meth treatment followed by 3 hours survival (n=8); or (3) meth treatment followed by 24 hours survival (n=8). Animals were euthanized by cervical dislocation at the indicated times.

To elucidate the role of ApoER2 and cytosolic signals in the regulation of ABC transporters and BBB integrity, a second set of mice received (1) vehicle injections (n=5); (2) meth injections (n=5), (3) meth injections combined with the ApoER2 inhibitor receptor-associated protein (RAP; 40 μg/kg; Bioworld, Dublin, OH) that was intraperitoneally administered on occasion of the last injection; this dose inhibits low-density lipoprotein receptor-related protein-8 without blocking low-density lipoprotein receptor[22] and does not influence lipid metabolism[23] (n=5); or (4) meth injections combined with the inducible nitric oxide synthase (iNOS) inhibitor 1400W (5 mg/kg; Sigma) that was intraperitoneally administered on occasion of each of the 3 meth injections (n=5). During the study, rectal temperature was measured with a rectal sonde. To test effects of pharmacological inhibitors alone in the absence of meth, additional mice received injections of (1) vehicle (n=4); (2) RAP (40 μg/kg; n=4); or (3) 1400W (5 mg/kg; n=4). Animals were euthanized 3 hours after the last injection. Brain cryostat sections were prepared at the level of the midstriatum (+0.8 mm of bregma) that were used for hematoxylin and eosin staining. The remaining striatum was dissected on ice.

To evaluate changes in arterial blood pressure, a third set of mice was anesthetized with urethane (1 g/kg, intraperitoneally) and placed on a homeothermic blanket (Harvard Apparatus). Urethane was chosen as anesthetic because among all known anesthetics urethane has least effects on blood pressure.[24] After systemic intraperitoneal delivery of urethane at doses comparable to those used previously, no changes in blood pressure have been reported.[25] Mean arterial blood pressure was recorded using a Laboratory-Trax-4/24T system (World Precision Instruments) through a polyethylene catheter that was implanted into the right femoral artery. One subset of animals was anesthetized 30 minutes before the first (1) vehicle (n=4); or (2) meth (n=5) injection. In these animals blood pressure values were taken at 6-minute intervals before and after each vehicle or meth injection over a duration of 30 minutes, resulting in a total of 5 measurements on occasion of each treatment. These animals were euthanized after the last recording on that same day. To examine longer-lasting effects of meth exposure, an additional subset of animals received 3 consecutive treatments with (1) vehicle (n=4); or (2) meth (n=5). These animals were anesthetized 24 hours after the last injection. During the next 24 minutes, a total of 4 blood pressure measurements were done, of which mean values were reported.

Microvessel Isolation and Protein Extraction

For total brain homogenates, tissue samples from animals belonging to the same group were pooled (n=4–8; see previously), homogenized, and lysated in 1% NP-40 buffer containing 50 mmol/L Tris-HCl and 150 mmol/L NaCl (pH 7.4) supplemented with 5% protease inhibitor cocktail and 1% phosphatase inhibitor cocktail-2 and sonicated. For microvessel isolation, pooled tissue samples were homogenized in microvessel isolation buffer and supplemented with 5% protease inhibitor cocktail and 1% phosphatase inhibitor cocktail-2. Homogenates were centrifuged at 3200 rpm for 10 minutes at 4°C. The resulting pellets were resuspended in 20% dextran (molecular weight 64 000–76 000; D4751; Sigma) in microvessel isolation buffer. Suspensions were centrifuged at 6500 rpm for 20 minutes at 4°C. The resulting crude microvessel-rich pellets were resuspended in microvessel isolation buffer and filtered through 2 nylon filters of 100-μm and 30-μm mesh size (Millipore, Schwalbach, Germany). Microvessels were stored at −80°C until further use. Isolated microvessels were homogenized in appropriate lysis buffers (see subsequently) supplemented with 5% protease inhibitor cocktail and 1% phosphatase inhibitor cocktail-2. Lysate samples were sonicated over 2 cycles lasting 20 seconds each at 4°C. Protein concentrations were measured using a Bradford assay kit with an iMark microplate reader (Bio-Rad, Hercules, CA). The microvessel fractions produced have previously been shown to exhibit a high degree of purity lacking glial fibrillary acidic protein, which is strongly expressed on astrocytic end feet.[20]

Western Blotting

Lysates containing 20 μg protein were complemented with 5× sodium dodecyl sulfate loading buffer. Samples were pretreated by heating and processed under reducing conditions except for the ABC transporter blots, for which nonheated samples were loaded under nonreducing conditions to avoid aggregation of these highly glycosylated membrane proteins.[20] Samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by Western blot analysis using primary antibodies diluted 1:100 for ABCC1 and 1:1000 for all other proteins in 5% skim milk and 0.1 mol/L Tris-buffered saline containing Tween 20%. Blots were digitized, densitometrically analyzed, and corrected for protein

Downloaded from http://ahajournals.org by on July 6, 2023

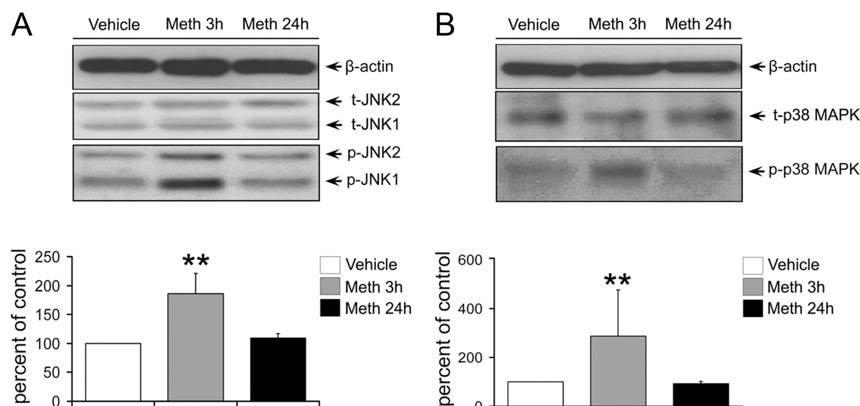

Figure 1. Methamphetamine induces brain parenchymal stress response. Western blot analysis using extracts from total brain homogenates showing that (**A**) JNK1/2 and (**B**) p38 MAPK phosphorylation (ie, activation) are transiently increased in the brain parenchyma at 3 hours after methamphetamine exposure. Note that total JNK1/2 and p38, which were evaluated with antibodies detecting both the nonphosphorylated and phosphorylated kinases, are not influenced by methamphetamine. Data are means±SD (n=4 Western blots). $^{*}P<0.05$/$^{**}P<0.01$ compared with vehicle. JNK indicates c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase.

loading by means of the β-actin or β-tubulin blots. Specifications of the antibodies used are given in the online-only Supplementary Materials and Methods.

Immunoprecipitation Assay

Lysates containing 800 μg protein that had been obtained using a 1% NP-40 lysis buffer containing 150 mmol/L NaCl and 50 mmol/L Tris base (pH 8.0) were supplemented with sodium orthovanadate (final concentration: 1 mmol/L) and complemented with 3 equal volumes of NET buffer (100 mmol/L Tris, 200 mmol/L NaCl, 5 mmol/L EDTA, 5% NP-40, pH 7.4).[20] Two micrograms of anti-ApoER2 antibody (Santa Cruz Biotechnology) were added to each sample and incubated overnight at 4°C under slight rotation. The next day 20 μL of protein A/G plus-agarose was added to the samples, which were incubated 1 hour at 4°C. Finally samples were centrifuged for 30 seconds at 15 000 rpm at 4°C. Supernatants were dispersed and pellets washed 3 times in ice cold NET buffer. Twenty microliters of 2×sodium dodecyl sulfate loading buffer was added to each pellet and boiled for 5 minutes followed by a short centrifugation at 4000 rpm to precipitate beads. Supernatants were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis using 10% acrylamide-bis gel followed by Western blot analysis for phosphotyrosine.

Immunohistochemistry for Leukocyte Marker CD45

Twenty-micron cryostat sections obtained from the midstriatum were incubated with 0.3% hydrogen peroxide in 70% methanol in Tris-buffered saline to block endogenous peroxidase. Sections were incubated overnight at 4°C with rat anti-CD45 antibody (1:20; BD Biosciences). The next day, sections were incubated with biotinylated goat antirat antibody (1:200; Santa Cruz Biotechnology). Immune reactions were revealed with diaminobenzidine tetrahydrochloride (Sigma) using a Vectastain AB kit (Vector Laboratories). Sections were evaluated under a microscope (Axioplan; Zeiss). CD45+ profiles were quantified in 2 regions of interest of the lateral striatum, which were 250 μm apart (each measuring 62 500 μm^2). Mean values were calculated for both areas. The striatum was selected because meth intoxication mainly affects the striatum.[1,3,4] In the latter study, sections of animals exposed to 30 minutes middle cerebral artery occlusion followed by 24 hours reperfusion served as positive controls.

Statistics

Statistical analysis was performed by using SPSS 17 for Windows. Data were evaluated by 1-way analysis of variance followed by least significant differences tests. For rectal temperature and blood pressure, baseline data and area-under-the-curve results were analyzed, because the number of measurements exceeded that of animals per group. Two-tailed tests, which were corrected for the number of comparisons, were then conducted as post hoc tests at individual time points. Results are presented as means±SD. Probability values <0.05 were considered significant.

Results

Methamphetamine Induces a Parenchymal Stress Response

To evaluate the effect of meth administration on the brain parenchyma, we analyzed the expression and activation of 2 stress activated kinases, JNK1/2 and p38 mitogen-activated protein kinase using antibodies detecting total (=nonphosphorylated and phosphorylated) and phosphorylated (ie, activated) JNK1/2 and p38. Interestingly, a robust increase in JNK1/2 (Figure 1A) and p38 (Figure 1B) phosphorylation was detected in total brain lysates at 3 hours after meth administration. This phosphorylation disappeared within 24 hours after meth exposure. In contrast to phosphorylated JNK1/2 and p38, total JNK1/2 and p38 were not influenced by meth (Figure 1A–B). Rectal temperature transiently increased after meth intoxication by approximately 1 to 2.5°C (online-only Supplemental Figure I). Mean arterial blood pressure was not influenced by meth exposure (online-only Supplemental Table I).

Methamphetamine Induces Adhesion Molecules in Cerebral Microvessels and Upregulates Tight Junction Proteins Without Provoking IgG Extravasation or Major Leukocyte Infiltration

To investigate how acute meth exposure influences vascular integrity, we studied the expression of adhesion molecules and tight junction proteins in cerebral microvessels and investigated the extravasation of the endogenous tracer IgG in total brain extracts, which upon BBB damage is able to accumulate in the brain parenchyma.[21] Meth transiently induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression (Figure 2A–B) and elevated occludin and claudin-5 expression at 3 but not 24 hours in cerebral microvessels (online-only Supplemental Figure IIA–B) but did not influence zonula occludens-1 levels (not shown). No IgG extravasation was noted in the brain parenchyma using Western blots (online-only Supplemental Figure IIC). Hematoxylin–eosin stainings revealed that the macroscopical structure of cerebral microvessels remained intact after meth exposure (online-only Supplemental Figure IID). Leukocyte counts in the stria-

Downloaded from http://ahajournals.org by on July 6, 2023

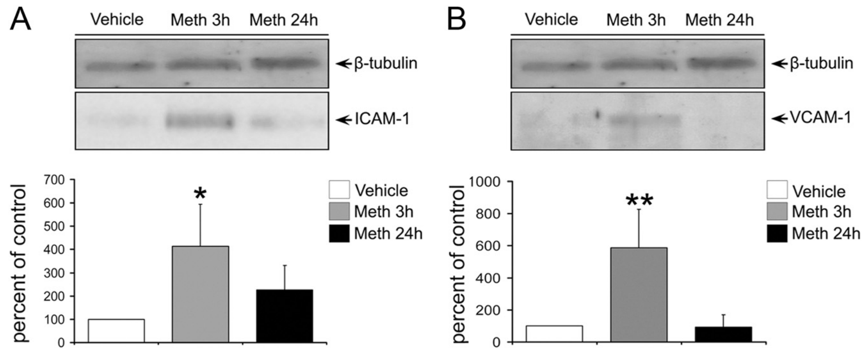

Figure 2. Methamphetamine induces cell adhesion molecules on cerebral microvessels. Western blot analysis using brain capillary extracts demonstrating that (**A**) ICAM-1 and (**B**) VCAM-1 are elevated at 3 hours after methamphetamine exposure. Note the reversal of ICAM-1 and VCAM-1 after 24 hours. Data are means±SD (n=4 Western blots). $^{*}P<0.05$/$^{**}P<0.01$ compared with vehicle. ICAM-1 indicates intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

tum, as evaluated by CD45 immunohistochemistry, were not significantly increased by meth (online-only Supplemental Figure III).

Methamphetamine Differentially Regulates ABCB1 and ABCC1 in Cerebral Microvessels

We next examined how meth influences the expression of the transporters ABCB1 and ABCC1 on cerebral microvessels. Interestingly, ABCB1, which is expressed on the luminal endothelial membrane,[18] was upregulated (Figure 3A), whereas ABCC1, which is localized on the abluminal endothelial membrane,[19] was downregulated (Figure 3B) at 3 hours after meth intoxication. ABCB1 and ABCC1 expression returned to baseline levels within 24 hours (Figure 3A–B).

ApoE Is Upregulated on Meth Exposure, ApoER2 Is Activated, and JNK1/2 and c-Jun Are Deactivated

Subsequent to brain ischemia, ApoE regulates ABCB1 and ABCC1 expression in an ApoER2/ JNK1/2/c-Jun-dependent way.[20] To investigate whether ApoE influenced ABC transporters after meth intoxication, we examined ApoE expression in total brain extracts, showing that ApoE was increased at 3 hours after meth exposure (Figure 4A). Subsequent analysis of microvessel extracts revealed that ApoER2 was activated by meth through tyrosine phosphorylation (Figure 4B). Concomitantly, JNK1/2 and c-Jun were dephosphorylated (ie, deactivated; Figure 4C–D).

Inhibition of ApoER2 and JNK1/2's Effector iNOS Re-Establish ABC Transporter Expression

To test whether ApoER2 was indeed responsible for mediating ABC transporter responses to meth exposure, we administered a pharmacological inhibitor of ApoER2, RAP, and an inhibitor of iNOS, 1400W. The iNOS inhibitor was chosen, because iNOS has previously been shown to act as effector of JNK1/2.[26,27] We found that either RAP or 1400W administration re-established ABCB1 and ABCC1 expression (Figure 5A–B) at the same time restoring JNK1/2 and c-Jun phosphorylation (Figure 5C–D). Rectal temperature was not influenced by RAP and 1400W (online-only Supplemental Figure I). RAP or 1400W alone did not change the expression of ABCB1 or ABCC1 (online-only Supplemental Figure IV).

Discussion

Using a mouse model of systemic meth intoxication, we show that acute exposure to meth at doses comparable to those used in drug addiction (doses ingested by consumers are typically in the range of 50–500 mg[28]) induces a transient parenchymal stress response, reflected by the activation of JNK1/2 and p38 mitogen-activated protein kinase, accompanied by the induction of proinflammatory cell adhesion molecules intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 on cerebral microvessels. Although tight junction proteins occludin, claudin-5, and zonula occludens-1 were expressed at a high level and microvascular permeability for the serum marker IgG was low, a differential regulation of ABC transporters, namely an upregulation of the luminal endothelial transporter ABCB1 and downregulation of the abluminal transporter ABCC1, was observed on cerebral microvessels, which, as we showed, was controlled by ApoE, most probably through its receptor ApoER2 that deactivated the JNK1/2/c-Jun pathway.

Cerebrovascular effects of meth have already been described previously. In rats and mice, acute meth exposure resulted in an increased permeability to endogenous and

Downloaded from http://ahajournals.org by on July 6, 2023

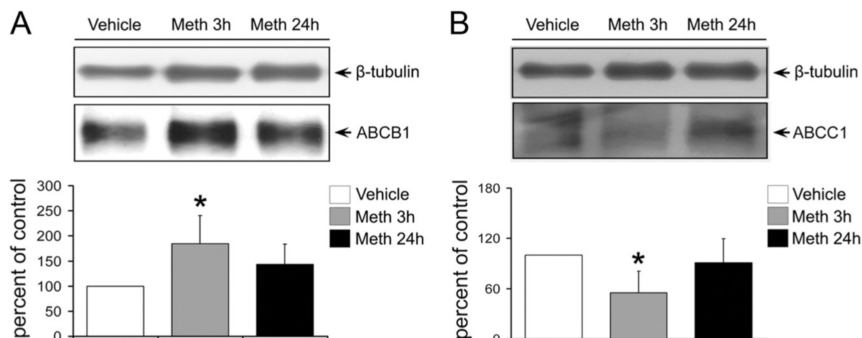

Figure 3. Methamphetamine differentially regulates ABCB1 and ABCC1 transporters. Western blot analysis using cerebral microvessel extracts showing that (**A**) ABCB1 expression is increased and (**B**) ABCC1 expression is decreased at 3 hours after methamphetamine intoxication. Note that ABCB1 and ABCC1 expression return to baseline levels after 24 hours. Data are means±SD (n=4 Western blots). $^{*}P<0.05$/$^{**}P<0.01$ compared with vehicle. ABC indicates adenosine triphosphate-binding cassette.

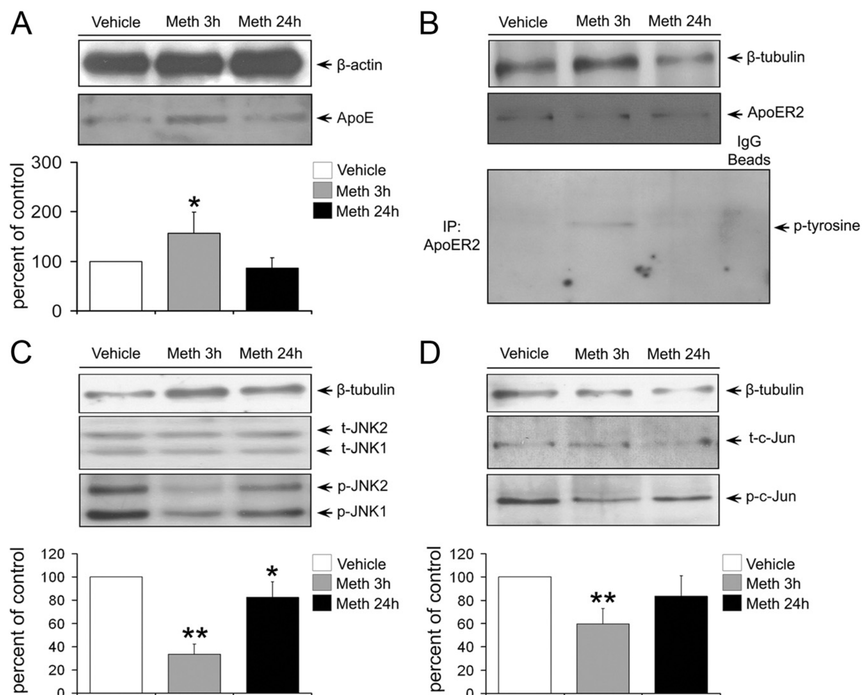

Figure 4. Methamphetamine increases ApoE expression, activating ApoER2 and deactivating JNK-1/2 and c-Jun in cerebral microvessels. Western blot analysis and immunoprecipitation studies using total brain (**A**) and cerebral microvessel (**B–D**) extracts showing that (**A**) ApoE is upregulated in the brain parenchyma by methamphetamine, activating (**B**) ApoER2 on brain capillaries through tyrosine phosphorylation and deactivating (**C**) JNK1/2 and (**D**) c-Jun by desphosphorylation. Note that total JNK1/2 and c-Jun are again not influenced by methamphetamine. Data are means±SD (n=4 Western blots). *$P<0.05$/**$P<0.01$ compared with vehicle. ApoE indicates apolipoprotein-E; ApoER2, apolipoprotein-E receptor-2; JNK, c-Jun N-terminal kinase.

exogenous BBB tracers.[8,9,29] However, such actions were observed at doses far above those consumed in drug addition associated with massive hyperthermia to more than 41.5°C suggesting subsequent lethality, whereas such changes were markedly attenuated under conditions of more subtle hyperthermia.[8,29] In addition, acute rises in arterial blood pressure lasting over several minutes were found after delivery of higher meth doses.[30,31] In 1 of those studies delivering meth at doses of 30 mg/kg, a decreased expression of the tight junction proteins occludin, claudin-5m and zonula occludens-1 was noted in the mouse hippocampus 24 hours after exposure.[9] We did not detect arterial blood pressure changes in the current study, and rectal temperature changes were only transient. We observed an elevated expression of occludin and claudin-5 and an unchanged expression of zonula occludens-1. IgG extravasation was low.

Despite absence of overt BBB breakdown, a differential regulation of ABC transporters was noted on cerebral microvessels. The luminal ABCB1, which carries its substrates in direction from the vessel into the blood,[18] was upregulated,

Downloaded from http://ahajournals.org by on July 6, 2023

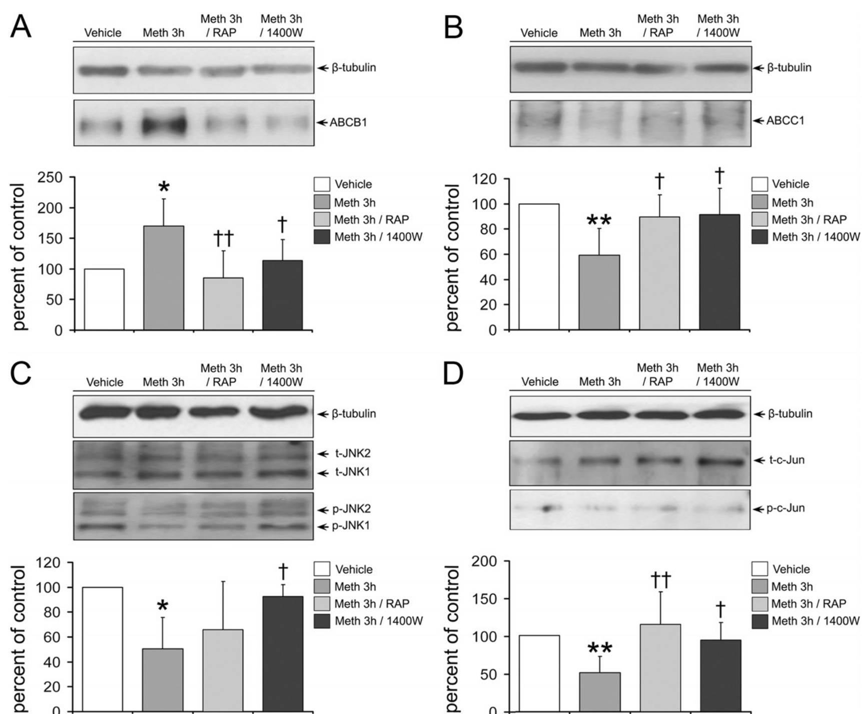

Figure 5. Inhibition of ApoER2 and iNOS re-establish ABC transporter expression. Western blot analysis using cerebral microvessel extracts showing that either the ApoER2 inhibitor RAP or the iNOS inhibitor 1400W restore (**A**) ABCB1 and (**B**) ABCC1 expression, increasing (**C**) JNK1/2 and (**D**) c-Jun phosphorylation (ie, activation). Data are means±SD (n=4 Western blots). *$P<0.05$/**$P<0.01$ compared with vehicle; †$P<0.05$/††$P<0.01$ compared with methamphetamine only. ApoER2 indicates apolipoprotein-E receptor-2; iNOS, inducible nitric oxide synthase; ABC, adenosine triphosphate-binding cassette; RAP, receptor-associated protein.

whereas the abluminal ABCC1, which carries its substrates in the opposite direction from the vessel into the brain,[19] was downregulated on brain capillary cells. The expression changes of ABC transporters were controlled by ApoE, which, as we further observed, activated its receptor ApoER2 on cerebral microvessels by tyrosine phosphorylation, thus dephosphorylating and deactivating JNK1/2 and c-Jun. We have previously shown in a model of transient focal cerebral ischemia that ApoE controls ABCB1 and ABCC1 expression on the transcriptional level through the JNK1/2/ c-Jun pathway.[20] In that study, we found that either genetic ApoE deletion or pharmacological JNK1/2 blockade prevented the physiological responses of ABC transporters.[20] We now complemented our previous findings with pharmacological ApoER2 and iNOS inhibition data, demonstrating that the ApoE/ApoER2/JNK1/2 pathway is similarly relevant for meth intoxication. Based on our results, ApoE that is released from the brain parenchyma might act as a sensor for brain injury in a variety of pathophysiological states, transmitting stress signals to the endothelial cells through ApoER2, which as we previously reported is constitutively expressed on the abluminal endothelial membrane.[20]

Through this ApoER2 signal, ABC transporters are regulated in a way that facilitates the removal of toxins from the brain tissue. We did not perform biodistribution experiments in the present study, yet we have previously shown in focal cerebral ischemia that alterations in ABCB1 and ABCC1 expression are accompanied by profound changes in the brain-to-blood distribution of ABC transporter substrates.[18,20] ABCB1 is a broad-spectrum transporter, which binds a large variety of environmental and also pharmacological compounds.[12] ABCB1 has previously been suggested to act as efflux transporter for meth at the murine BBB.[17] Compared with ABCB1, ABCC1 has more restricted substrate-binding properties.[19] ABCC1 was shown to have strong affinity to amphipathic molecules and brain metabolites among those Phase II degradation products generated as a consequence of oxidation processes.[32] At the murine BBB, such metabolites were shown to enter the brain parenchyma across the ABCC1 transporter.[19] Meth is extensively metabolized, generating large amounts of reactive oxygen species, including nitric oxide and peroxynitrates in the brain.[4,5] These metabolites require detoxification and removal across the BBB. By upregulating the luminal ABCB1 and downregulating the abluminal ABCC1 transporter, the brain possesses an efficient detoxification mechanism. Strengthening this ABC transporter response might represent a promising strategy that might allow the prevention of meth-induced brain injury.

Downloaded from http://ahajournals.org by on July 6, 2023

Acknowledgments

We thank Beate Karow for technical assistance.

Sources of Funding

Supported by the German Research Foundation (HE3173/2-1 and HE3173/3-1 to D.M.H.), Dr-Werner-Jackstädt Foundation (to A.EA.), Heinz-Nixdorf Foundation (to D.M.H.), Spanish Ministry of Science and Innovation (SAF2010-21529 to M.I.C.), Plan Nacional Sobre Drogas (PR47/10-17826 to M.I.C.), Red de Trastornos Adictivos (RD06/0001/006 to M.I.C.), and UCM (910258; to M.I.C.). A.U. received funding from Spanish Ministry of Science and Innovation, and A.R.-A. from Red de Trastornos Adictivos.

Disclosures

None.

References

1. Sandoval V, Hanson GR, Fleckenstein AE. Methamphetamine decreases mouse striatal dopamine transporter activity: roles of hyperthermia and dopamine. *Eur J Pharmacol.* 2000;409:265–271.
2. Sugimoto K, Okamura K, Tanaka H, Takashima S, Ochi H, Yamamoto T, et al. Methamphetamine directly accelerates beating rate in cardiomyocytes by increasing Ca(2+) entry via L-type Ca(2+) channel. *Biochem Biophys Res Commun.* 2009;390:1214–1220.
3. Xie T, McCann UD, Kim S, Yuan J, Ricaurte GA. Effect of temperature on dopamine transporter function and intracellular accumulation of methamphetamine: implications for methamphetamine-induced dopaminergic neurotoxicity. *J Neurosci.* 2000;20:7838–7845.
4. Kuhn DM, Geddes TJ. Molecular footprints of neurotoxic amphetamine action. *Ann N Y Acad Sci.* 2000;914:92–103.
5. Cadet JL, Jayanthi S, Deng X. Speed kills: cellular and molecular bases of methamphetamine-induced nerve terminal degeneration and neuronal apoptosis. *FASEB J.* 2003;17:1775–1788.
6. Sekine Y, Ouchi Y, Sugihara G, Takei N, Yoshikawa E, Nakamura K, et al. Methamphetamine causes microglial activation in the brains of human abusers. *J Neurosci.* 2003;28:5756–5761.
7. Sriram K, Miller DB, O'Callaghan JP. Minocycline attenuates microglial activation but fails to mitigate striatal dopaminergic neurotoxicity: role of tumor necrosis factor-alpha. *J Neurochem.* 2006;96:706–718.
8. Kiyatkin EA, Brown PL, Sharma HS. Brain edema and breakdown of the blood–brain barrier during methamphetamine intoxication: critical role of brain hyperthermia. *Eur J Neurosci.* 2007;26:1242–1253.
9. Martins T, Baptista S, Gonçalves J, Leal E, Milhazes N, Borges F, et al. Methamphetamine transiently increases the blood–brain barrier permeability in the hippocampus: role of tight junction proteins and matrix metalloproteinase-9. *Brain Res.* 2011;1411:28–40.
10. Ho EL, Josephson SA, Lee HS, Smith WS. Cerebrovascular complications of methamphetamine abuse. *Neurocrit Care.* 2009;10: 295–305.
11. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte–endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci.* 2006;7:41–53.
12. Löscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. *Nat Rev Neurosci.* 2005;6:591–602.
13. Hermann DM, Kilic E, Spudich A, Krämer SD, Wunderli-Allenspach H, Bassetti CL. Role of drug efflux carriers in the healthy and diseased brain. *Ann Neurol.* 2006;60:489–498.
14. Mahajan SD, Aalinkeel R, Sykes DE, Reynolds JL, Bindukumar B, Adal A, et al. Methamphetamine alters blood–brain barrier permeability via the modulation of tight junction expression: implication for HIV-1 neuropathogenesis in the context of drug abuse. *Brain Res.* 2008;1203: 133–148.
15. Ramirez SH, Potula R, Fan S, Eidem T, Papugani A, Reichenbach N, et al. Methamphetamine disrupts blood–brain barrier function by induction of oxidative stress in brain endothelial cells. *J Cereb Blood Flow Metab.* 2009;29:1933–1945.
16. Abdul Muneer PM, Alikunju S, Szlachetka AM, Murrin LC, Haorah J. Impairment of brain endothelial glucose transporter by methamphetamine causes blood–brain barrier dysfunction. *Mol Neurodegener.* 2011;6:23.
17. Mann H, Ladenheim B, Hirata H, Moran TH, Cadet JL. Differential toxic effects of methamphetamine (METH) and methylenedioxymethamphetamine (MDMA) in multidrug-resistant (mdr1a) knockout mice. *Brain Res.* 1997;769:340–346.
18. Spudich A, Kilic E, Xing H, Kilic U, Rentsch KM, Wunderli-Allenspach H, et al. Inhibition of multidrug resistance transporter-1 facilitates neuroprotective therapies after focal cerebral ischemia. *Nat Neurosci.* 2006; 9:487–488.
19. Kilic E, Spudich A, Kilic U, Rentsch KM, Vig R, Matter CM, et al. ABCC1: a gateway for pharmacological compounds to the ischaemic brain. *Brain.* 2008;131:2679–2689.
20. ElAli A, Hermann DM. Apolipoprotein-E controls ATP-binding cassette transporters in the ischemic brain. *Sci Signal.* 2010;3:ra72.
21. ElAli A, Hermann DM. Liver X receptor activation enhances blood-brain barrier integrity in the ischemic brain and increases the abundance of

ATP-binding cassette transporters ABCB1 and ABCC1 on brain capillary cells. *Brain Pathol.* Published ahead of print July 18, 2011. DOI: 10.1111/j.1750-3639.2011.00517.x.
22. Yu KC, Jiang Y, Chen W, Cooper AD. Evaluation of the components of the chylomicron remnant removal mechanism by use of the isolated perfused mouse liver. *J Lipid Res*. 1999;40:1899–1910.
23. Lambert MP, Wang Y, Bdeir KH, Nguyen Y, Kowalska MA, Poncz M. Platelet factor 4 regulates megakaryopoiesis through low-density lipoprotein receptor-related protein 1 (LRP1) on megakaryocytes. *Blood*. 2009;114:2290–2298.
24. Maggi CA, Meli A. Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 2: cardiovascular system. *Experientia*. 1986;42:292–297.
25. Bell GJ, Hiley CR, Yates MS. The effect of four general anesthetic agents on the regional distribution of cardiac output in the rat. *Br J Pharmacol*. 1977;61:126–127.
26. Lahti A, Jalonen U, Kankaanranta H, Moilanen E. c-Jun NH2-terminal kinase inhibitor anthra(1,9-cd)pyrazol-6(2H)-one reduces inducible nitric-oxide synthase expression by destabilizing mRNA in activated macrophages. *Mol Pharmacol*. 2003;64:308–315.
27. Pawate S, Bhat NR. C-Jun N-terminal kinase (JNK) regulation of iNOS expression in glial cells: predominant role of JNK1 isoform. *Antioxid Redox Signal*. 2006;8:903–909.
28. Cruickshank CC, Dyer KR. A review of the clinical pharmacology of methamphetamine. *Addiction*. 2009;104:1085–1099.
29. Bowyer JF, Robinson B, Ali S, Schmued LC. Neurotoxic-related changes in tyrosine hydroxylase, microglia, myelin, and the blood–brain barrier in the caudate–putamen from acute methamphetamine exposure. *Synapse*. 2008;62:193–204.
30. Wang Y, Hayashi T, Chang CF, Chiang YH, Tsao LI, Su TP, Borlongan C, Lin SZ. Methamphetamine potentiates ischemia/reperfusion insults after transient middle cerebral artery ligation. *Stroke*. 2001;32:775–782.
31. Polesskaya O, Silva J, Sanfilippo C, Desrosiers T, Sun A, Shen J, Feng C, Polesskiy A, Deane R, Zlokovic B, Kasischke K, Dewhurst S. Methamphetamine causes sustained depression in cerebral blood flow. *Brain Res*. 2011;1373:91–100.
32. Hermann DM, Bassetti CL. Implications of ATP-binding cassette transporters for brain pharmacotherapies. *Trends Pharmacol Sci*. 2007;28:128–134.

Downloaded from http://ahajournals.org by on July 6, 2023