Temperature 1:3, 172–182; October/November/December 2014

Amphetamine- and methamphetamine-induced hyperthermia: Implications of the effects produced in brain vasculature and peripheral organs to forebrain neurotoxicity

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Keywords: hyperthermia, neurotoxicity, amphetamine, methamphetamine, cerebral vasculature, meninges, blood-brain barrier, immune system

Abbreviations: AMPH, amphetamine; BBB, blood-brain barrier; CBF, cerebral blood flow; CSF, cerebrospinal fluid; EIH, environmentally-induced hyperthermia; LPS, lipopolysaccharides; METH, methamphetamine; MAV, meninges and associated vasculature; ROS, reactive oxidative stress.

The adverse effects of amphetamine- (AMPH) and methamphetamine- (METH) induced hyperthermia on vasculature, peripheral organs and peripheral immune system are discussed. Hyperthermia alone does not produce amphetamine-like neurotoxicity but AMPH and METH exposures that do not produce hyperthermia (≥40°C) are minimally neurotoxic. Hyperthermia likely enhances AMPH and METH neurotoxicity directly through disruption of protein function, ion channels and enhanced ROS production. Forebrain neurotoxicity can also be indirectly influenced through the effects of AMPH- and METH- induced hyperthermia on vasculature. The hyperthermia and the hypertension produced by high doses amphetamines are a primary cause of transient breakdowns in the blood-brain barrier (BBB) resulting in concomitant regional neurodegeneration and neuroinflammation in laboratory animals. This BBB breakdown can occur in the amygdala, thalamus, striatum, sensory and motor cortex and hippocampus. Under these conditions, repetitive seizures greatly enhance neurodegeneration in hippocampus, thalamus and amygdala. Even when the BBB is less disrupted, AMPH- or METH- induced hyperthermia effects on brain vasculature may play a role in neurotoxicity. In this case, striatal and cortical vascular function are adversely affected, and even greater ROS, immune and damage responses are seen in the meninges and cortical surface vasculature. Finally, muscle and liver damage and elevated cytokines in blood can result when amphetamines produce hyperthermia. Proteins, from damaged muscle may activate the peripheral immune system and exacerbate liver damage. Liver damage can further increase cytokine levels, immune system activation and increase ammonia levels. These effects could potentially enhance vascular damage and neurotoxicity.

Earlier History of Amphetamine- and Methamphetamine-Induced Hyperthermia and Neurotoxicity

Introductory remarks

The terms amphetamine (AMPH) and methamphetamine (METH) neurotoxicity are often used interchangeably in this review. This is because research from our laboratory has not been able to identify any appreciable difference in the neurotoxicity produced in rodent by AMPH compared to METH.[1,2] **Figure 1** shows the profiles of the temperature changes produced by either exposure to amphetamines or environmentally-induced hyperthermia (EIH) that we have observed in our laboratory. The interactive effects that hyperthermia has with respect to toxicity during either AMPH or METH exposures are complex. **Table 1** summarizes the physiological and pathological effects that can be produced by 1) EIH, 2) AMPH or METH exposures producing significantly hyperthermic conditions or 3) AMPH or METH exposures when life-threatening hyperthermia occurs. **Table 2** provides information on the role of selected biochemical

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Submitted: 09/29/2014; Revised: 10/22/2014; Accepted: 10/27/2014

http://dx.doi.org/10.4161/23328940.2014.982049

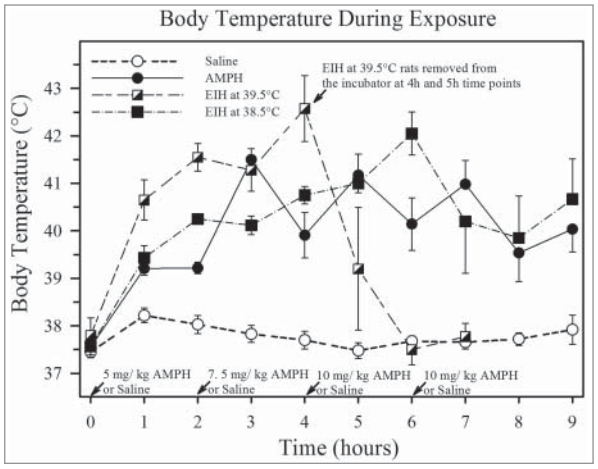

Figure 1. The effect of amphetamine (AMPH) on body temperature compared to environmentally-induced hyperthermia (EIH) and normothermic controls. The results of one of the more recent studies in the authors' laboratory compares the hyperthermia observed during neurotoxic exposures to AMPH with that produced by EIH, which is very similar to heat stroke. The temperature profiles of animals given either 4 doses of either AMPH (n = 10) or normal saline (normothermic controls n = 9) s.c. at an environmental temperature of 22.5°C are shown. Their temperature profiles are compared to 2 groups of animals given 4 doses of saline in an infant incubator held at either 38°C to 39°C (ave. ≈38.5, n = 6) or 39°C to 40°C (ave. ≈39.5, n = 4) which induced hyperthermia (EIH). Animals at the higher incubator temperature became hyperthermic much more rapidly and as a group had slightly higher peak temperatures. They all had ataxia and hind limb dysfunction for 2 to 8 h after cooling. The second group of EIH animals at the lower 38.5°C temperature had a temperature profile almost identical to the AMPH group. The variability of the body temperatures of the AMPH group and the 38.5°C EIH group after the 3 h time point was due to cooling on ice to prevent death. The 39.5°C EIH group was not subjected to any further hyperthermia after 4 h since it would have been lethal (previous studies in the authors' laboratory).

or physiological effects mediated by hyperthermia that are associated with AMPH and METH neurotoxicity. The reader can use these tables as adjunct references where they pertain to what is being presented in the text.

Striatal dopamine terminal damage

Starting in the early 1960s, clinical reports and research in laboratory animals began to point to the important role that hyperthermia had in exacerbating the adverse physiological and lethal effects induced by AMPH and METH in humans [3-5] and laboratory animals.[4,6,7] Approximately 10 years later, reports of METH damaging dopaminergic terminals were published.[8-13] However, it wasn't until the early 1990s that the role of hyperthermia in METH and AMPH neurotoxicity began to be elucidated. At that time, the link between dopamine terminal degeneration in the striatum and pronounced hyperthermia (≥40°C) was reported in both rat [14-16] and mouse.[17,18] It is now clear that when animals remain normothermic during exposures to very high doses of AMPH or METH, more transient depletions of striatal dopamine (decreases of 40 to 60% in mouse and 45 to 65% in rat) lasting for 1 month or so result along with rare sporadic occurrences of neurodegeneration in the parietal and piriform cortex.[19-22] Under these normothermic conditions there is minimal or no neuroimmune response in the striatum. However, it was noted that pronounced hyperthermia alone would not produce this neurotoxicity. Under favorable conditions (waking cycle and in a 23.5°C environment) and with 8 h exposures, plasma concentrations of AMPH or METH as low as 3 μM are capable of producing hyperthermia and neurotoxicity.[2,23] Further research in non-human primates has helped substantiate the clinical relevance of the hyperthermia in regards to METH producing dopamine terminal degeneration.[24,25]

METH-induced hyperthermia and excessive plasma membrane transporter (DAT) activity appear to be primary factors in the production of this striatal neurotoxicity.[26] However, reactive metabolites of dopamine,[27,28] vesicular monoamine transporter-2 damage,[29] and elevated glutamate levels[30-34] have also been implicated as exacerbating factors in METH toxicity. Increases in body and brain temperatures during AMPH exposure do appear to correlate with increased striatal dopamine and amygdala 5HT levels.[15,29,35,36] Finally, the great swelling and loss of Fluoro-Ruby labeled axons of dopaminergic neurons in the striatum indicate that they are being destroyed by a "necrotic"-like effect.[37] The exact mechanism of the apparent dopaminergic axonal and terminal destruction is unknown but is not due to classic apoptosis or necrosis since the loss of dopaminergic neurons in the substantia nigra is minimal at best (≤20%) compared to the ≥80% loss of striatal dopamine terminals.[38]

There are several mechanisms by which hyperthermia could potentiate AMPH and METH toxicity to dopamine terminals that have also been implicated in other types of neuronal degeneration. METH-induced hyperthermia directly increases ROS levels in striatum and causes a dramatic increase in ROS-induced gene expression.[39-42] Interestingly, hyperthermia (EIH) alone produces equivalent increases in genes up-regulated by ROS in many brain regions.[43] Concomitant with large increases in ROS and heat-shock protein induction is the dysfunction of proteins due to misfolding produced by pronounced hyperthermia.[44,45] Such protein alterations or changes in lipid membranes could lead to mitochondrial[46] and ion channel dysfunction.[47-52] The tremendous increase in DAT activity/ transport produced by AMPH or METH may be sufficient to produce detectable depolarization due to concomitant Na^+ influx with either AMPH or METH amphetamine into dopamine terminals and possibly alter glutamate activity.[33,53] These affects will lead to large increases in sodium into the terminal that will require extensive amounts of energy to transport it out of the terminal. This alone is not sufficient to produce terminal degeneration without hyperthermia. However, we postulate that the occurrence of hyperthermia in the presence of AMPH or METH further compromise ion channels, mitochondrial function and damage to other import cellular components by ROS leading to terminal damage and/ or death.

Neuronal degeneration in forebrain

Specialized histological techniques and the knowledge that hyperthermia during exposures to amphetamines was necessary for dopamine terminal damage were applied to laboratory animal

Table 1. Effects of hyperthermia alone (EIH) compared to the toxicity of AMPH or METH exposures that are produced when hyperthermia occurs

	Exposure Group		
Physiological or Pathological Effect	EIH	AMPH or METH with 40°C ≤ Body Temp. < 41°C	AMPH or METH with 41.0°C ≤ Body Temp. < 43.0°C
Dopamine Terminal Damage in Striatum	None	50% < Depletion ≤ 80%	80% < Depletion ≤ 95%
**Parietal Cortex Neurodegeneration	None	Present but diffuse	More prevalent at these body temperature ranges
Limbic Cortex Neurodegeneration	minimal	Present but diffuse	Extensive if seizures occur
Thalamus Neurodegeneration	None	Present but diffuse	More extensive at these body temp. ranges
Hippocampal Neurodegeneration	None	Minimal in rats but can be	*Extensive if motor seizure activity occurs
Convulsions/ Behavioral Seizures	**None	Convulsions often occur in mice but not in rats	Convulsions and status epilepticus
BBB disruption	Yes	Not in rat *but possibly in mice	*Yes
Choroid Plexus Dysfunction/ Damage	Yes	Not determined	≤ EIH
[a]MAV Dysfunction/ Damage	Yes	Not determined	> EIH
Elevated Serum **Myoglobin	Increase < 2-fold	2-fold < Increase < 3-fold	3-fold < Increase < 10-fold
Elevated Serum **Bound Urea Nitrogen	Increase ≈ 2-fold	Increase < 2-fold	2-fold < Increase < 3-fold
Elevated Serum **Alanine Transaminase	***1-fold < Increase < 10-fold	< 2-fold	Increase ≈ 4-fold
Blood Glucose	≈ Normal 100 to 150 mg/ dL	60 to 100 mg/ dL	30 to 80 mg/ dL
Peripheral Immune System Changes	Yes	Yes	Yes

Note that this table represents a summary of the findings of the authors' laboratory or (in a few instances) several other investigators. The damage estimates shown in the table for the given peak body temperature ranges with AMPH or METH are when these body temperatures are maintained for a duration of ≈ 3 h or more. Limbic cortex areas evaluated are piriform and the amygdala cortices.

[a] MAV is an abbreviation for meninges and associated cerebral cortical vasculature (includes all major cortical surface vasculature as well as pial arterioles).

*Damage is seizure dependent and is more prevalent with very high (> 20 mg/ kg) doses of AMPH or METH. It should be noted that overt motor seizures/ convulsions may not be necessary for neurodegeneration but that electrographic signs of epileptoid activity without convulsions is sufficient (from communications with Dr. Denson Fujikawa).

**Pertains to rat data.

***Effects strain dependent.

research starting in the mid-1990s. These studies resulted in the identification of brain regions where METH and AMPH produced cell body/ somatic neurodegeneration. The use of Fluoro-Jade to label degenerating neurons after AMPH and METH exposure in histological preparations of forebrain was key for the success of these studies in the author's and other laboratories.[54-57] Areas of the somatosensory parietal cortex (vibrissae input) and limbic system (piriform and amygdala cortex and tenia tecta) were most sensitive. However, in animals in which the most pronounced (≥ 41.5°C) and prolonged hyperthermia was produced by AMPH, neurodegeneration was more extensive in the intralaminar regions of the thalamus and striatum.[54] Hippocampal degeneration was minimal unless seizures occurred in animals. Neurotoxicity in the striatum, neocortex, and limbic regions of human brain has been reported with METH abuse.[58-64] For a more complete description of how neurotoxic dosing regimens of AMPH or METH interact with body temperature and seizures to produce neurodegeneration in various brain regions, see Bowyer et al. 2008.[1] It is still not known the degree to which body temperatures must be increased by AMPH or METH to produce dopamine terminal damage and neurodegeneration in humans.

The neurodegeneration observed in AMPH or METH animal studies[65] using lower doses which do not usually produce seizure activity was restricted to the parietal cortex, and it was significantly less than that produced by systemic administration of either kainic or domoic acid (limbic cortex, hippocampus and cortex)[66-68] or 3-nitroproprionic acid (striatum and thalamus).[69-71] However, starting in 1998 more pronounced types of neurodegeneration found in limbic cortex, hippocampus and thalamus were observed in rats given multiple doses of 15 mg/kg AMPH [54] and particularly mice given single high doses (40 mg/ kg) of METH.[72] The most likely explanation at the time was that these types of AMPH or METH exposures produced high/ neurotoxic levels of glutamate in the synaptic cleft or that ion channel dysfunction occurred due to METH-induced hyperthermia. However, there are other explanations.

Role of the Brain Vasculature in Neurodegeneration Produced by AMPH and METH

Overt BBB disruption and vascular leakage

We observed in 1998 that vasculature damage might be related to some types of AMPH-induced neurodegeneration. In some instances, exposure to multiple doses of AMPH over an 8 h period could, in conjunction with extreme hyperthermia, produce perivascular neurodegeneration in the thalamus and hippocampus.[54] Subsequently, the studies by Deng et al. [72] indicated that a single very high dose of METH in mice could

Table 2. The role of selected biochemical or physiological effects mediated by hyperthermia that are associated with AMPH and METH neurotoxicity

	Adverse Biochemical or Physiological Effects Associated with Amphetamine or Methamphetamine Neurotoxicity						
Brain Region and Effect	**Reactive Oxidative Stress**	**Elevated Extracellular Glutamate**	**Hyperactivity of Dopamine Transporter**	**Blood-Brain Barrier Disruption**	**Significant Seizure Activity**	**Meninges and Associated Vasculature**	**Neuro-inflammation**
↓Striatal Dopamine Axons/ Terminals	Strong Supporting Data	Significant Supporting Data	Strong Supporting Data	Not Necessary	Not Necessary	Not Necessary	**Not Necessary?
Striatal Neurodegeneration	Supporting Data	Supporting Data	*Indirect	Sparse Supporting Data	Unknown	Not Necessary	Unknown
Parietal Neurodegeneration	Supporting Data	Supporting Data	*Indirect	Not Necessary	Not Necessary	Some Supporting Data	Unknown
Piriform Neurodegeneration	Some Supporting Data	Indirect Supporting Data	*Indirect	Supporting Data	Supporting Data	Some Supporting Data	Unknown
Thalamic Neurodegeneration	Some Supporting Data	Unknown	*Indirect	Supporting Data	Not Necessary	Unknown	Unknown
Amygdala Neurodegeneration	Some Supporting Data	Indirect Supporting Data	*Indirect	Supporting Data	Supporting Data	Unknown	Unknown
Hippocampal Neurodegeneration	Some Supporting Data	Indirect Supporting Data	*Indirect	Supporting Data	Significant Supporting Data	Unknown	Unknown

All the adverse biochemical or physiological effects listed are temperature dependent or greatly exacerbated by hyperthermia. The neurotoxicities listed are those that have been characterized histologically. The conclusions shown in the table are opinions of the authors derived from the literature. Most of which are supported to some extent by citation in the text.

Even though **systemic immune system activation** and **peripheral organ damage** all precede and correlate very positively with AMPH and METH toxicity, their roles at this point are still being investigated and controversial.

[a]Dopamine plasma membrane (a.k.a. DAT, *Slc6a*)

*Almost all of the hyperthermic and neurotoxic effects of AMPH and METH are either directly or indirectly due to very high synaptic extracellular levels of dopamine or norepinephrine.

**See text for clarification.

produce a much more pronounced neurodegeneration in the hippocampus and striatum than that observed in previous experiments evaluating METH and AMPH neurotoxicity. We speculated that vascular damage and repetitive seizures (status epilepticus) might play a role in such a very high dose effect, and later experiments bore this out to be true with METH and AMPH.[1,19] These single, very high doses produced a rapid and pronounced onset of hyperthermia and subsequently status epilepticus resulting in a consistent breakdown of the BBB in the amygdala, hippocampus and, in some instances, the striatum. The neurodegeneration that subsequently occurs in these areas approaches that produced by systemic kainic or domoic acid in regards to the number (thousands) of degenerating neurons observable in a 30 to 40 μm coronal sections seen in these regions.

Others have reported that more moderate doses of METH and AMPH could produce localized damage to the BBB in several brain regions when hyperthermia occurred, and indicated that hyperthermia induced by EIH (in a 38° to 40°C environment) alone could also result in BBB disruption and neurotoxicity.[73-75] As well, the combination of stress and METH appear to exacerbate more moderate types of damage to vasculature.[76] Our research involving AMPH and METH has consistently indicated that dopamine terminal damage could not be produced by EIH alone.[1,14,54] As well, diffuse neurodegeneration (somatic) in areas of the brain, such as parietal and piriform cortex, were not observed with EIH. Although studies in our laboratory indicate that hyperthermia alone (EIH) produces BBB disruption, we have observed that the pattern of the neurodegeneration and magnitude that accompanies the disruption is much less than that seen with AMPH and METH.[1] In summary, there is substantial evidence that, when METH and AMPH produced extreme hyperthermia, regional BBB breakdowns can occur, which greatly enhance neurotoxicity. Such a disruption would likely prevent the regulation of the composition of the extracellular constituents surrounding neurons in affected regions, which could enhance seizure activity and neurodegeneration by most mechanisms proposed to be involved in neurotoxicity (e.g., ROS and excitotoxicity). It should be noted that the BBB disruption that occurs after either AMPH or METH are normally rapidly reversed, within 2 h after the end of hyperthermia ($\leq$ 40°C), except in cases where extensive hippocampal neurodegeneration has occurred.[1,19,77]

Adverse effects of hyperthermia and amphetamines on choroid plexus, meninges and cerebral surface vasculature: a role in the exacerbation of neurotoxicity

One mechanism by which AMPH and METH may exacerbate neurodegeneration observed in the cortical regions (e.g., parietal or piriform cortex) of rat is through ischemia (decrease in cerebral blood flow, CBF) produced by vasoconstriction. This would result from AMPH and METH directly releasing norepinephrine from the noradrenergic innervation regulating the α1 noradrenergic receptors on pial arteries,[78-80] and the resulting vasoconstriction reducing CBF in the cortex. The locus coeruleus is the other major noradrenergic input associated with brain that directly innervates cortex.[81,82] This input has been shown to play a role in regulating cortical blood flow,[83] and has often been associated with global decreases in CBF,[84-86] which when combined with pial artery constriction would further increase the likelihood of cortical ischemia. However, more recent studies indicate that locus coeruleus input into parietal cortex may actually increase CBF through α and β noradrenergic receptors COX-2 and GABAergic cortical neurons and reduce ischemia.[87]

Thus, the overall net effect of AMPH and METH on CBF under neurotoxic and vasculotoxic conditions might not be easily predicted. None the less, one animal study clearly showed that METH was clearly capable of suppressing CBF during METH exposure and even after METH levels had subsided.[88] Furthermore humans abusing amphetamines can develop cerebral vascular accidents and have worse outcomes than those not abusing amphetamines.[89-91] Hypoxia lasting 24 h after exposure is also induced in laboratory animals by high doses (8 mg/ kg i.v.) of METH [92]; however, the degree of hyperthermia and convulsive activity was not reported. Clearly, AMPH- and METH-induced vasospasms and the ischemia thus produce would be a factor that could contribute to cortical neurodegeneration.

There is indication at the mRNA transcript level that regulation of vascular tone, and possibly damage, in the striatum and parietal cortex is somewhat altered by more moderate neurotoxic exposures to AMPH and METH (those not producing repetitive seizures or BBB leakage).[43,93] The slight (<2-fold) increases in mRNA for endothelial nitric oxide synthase (*Nos3*) and endothelin 1 (*Edn1*) would be expected if ischemia or vascular endothelial damage was occurring. The increase in *Nos3* may be a response mechanism to produce more nitric oxide and reverse any maladaptive vasoconstriction present. The insult produced by either AMPH or EIH to vasculature present in the choroid plexus and the meninges and associated cerebral vasculature (MAV) appears to be significantly greater.[43,93] This effect could be reflected in humans by the interaction of METH abuse and the development of meningitis, which has been reported.[94]

Also, there are many more pronounced increases in genes related to the immune system and inflammation in the choroid plexus and MAV (even more so) after AMPH or EIH. The gene expression changes in the MAV indicate that the lingering effects of AMPH damage coincide and may induce the vasospasms and prolonged decrease in CBF in the cortex discussed in the previous paragraph. Large increases in lipopolysaccharide (LPS) binding protein mRNA (*Lbp*) are observed in the choroid plexus and MAV at 1 day after AMPH but not EIH. *Lbp* increases are not seen in the parietal cortex and striatum after AMPH. Thus, in MAV and choroid plexus, *Lbp* increases are a unique immune response, which may be related to vascular damage to the MAV.[43] LPS binding protein is an important part of the innate immune response, is a biomarker for sepsis and has been reported to bind LPS from bacteria.[95,96] Whether the dramatic increase of *Lbp* in MAV after neurotoxic exposures to AMPH indicates an increased presence of bacteria (presages sepsis?) or activation of the innate immune system by other mechanisms remains to be determined.

In regards to the choroid plexus, previous studies indicate that AMPH does not appear to affect it, with regards to vascular and secretory cell damage, but that EIH is very detrimental to the choroid plexus.[97,98] Data from our laboratory showed a greater effect of AMPH on the choroid plexus then that reported by others.[43] Differences seen between the physiological effects of AMPH in our study with those reported in the earlier study, may explain the greater adverse effects. That is, more prolonged neurotoxic exposure to AMPH produced both severe hyperthermia and hypertension which was not observed in the earlier study with a single dose of AMPH.[98] In addition, adverse effects produced by hyperthermia alone (EIH) are likely as great, or greater, than AMPH.[43] Damage to the vascular and secretory cells present in the choroid plexus in a previous study involving hyperthermia or hypertension have resulted in neurotoxicity involving some neurodegeneration.[97] However, our findings over the years have found that extreme, even to a greater degree than that produced by AMPH or METH; hyperthermia alone (EIH) does not produce the histological signs of neurodegeneration resembling AMPH or METH neurotoxicity.

Indirect Adverse Effects on Vasculature Due to the Muscle, Liver in Kidney Damage Produced by Hyperthermia and Amphetamines

The linkage between amphetamines and rhabdomyolosis, which produces muscle damage, goes back over 40 years.[99-102] Also, the correlation between the magnitude of hyperthermia, serum myoglobin levels (resulting from muscle damage) and neurotoxicity produced by AMPH is very strong [103] as is the correlation between myoglobin and kidney damage.[104,105] However, this is not proof that myoglobin levels are necessarily a causative effect in AMPH neurotoxicity. An equivalent hyperthermia produced by EIH resulted in a lesser non-statistically significant increase in myoglobin. When hemoglobin is released during hemolysis it can cause vascular toxicity.[106,107] One would suspect that myoglobin, which like hemoglobin is heme containing and binds oxygen, may also be vasculotoxic. However, there is surprisingly little in the literature regarding the relationship between myoglobin in the circulating blood and toxicity to vasculature endothelium.

Also, there is some correlation between neurotoxic AMPH exposures with respect to blood nitrogen (BUN) but EIH can also produce similar significant increases in BUN indicating it is necessarily dependent on exposure to neurotoxic doses of AMPH.[103] The muscle damage produced by neurotoxic doses of AMPH, when hyperthermia occurs, also results in increased circulating concentrations of other enzymes such as creatine kinase.[103] Both the creatine kinase and myoglobin increases (5- to 6-fold control) were more pronounced than BUN levels (2- to 3-fold control). Although the increase in myoglobin and creatine kinase in blood could be due to renal damage, we found no evidence of renal damage histologically. Therefore, it is a plausible that significant increases in many types of muscle-related proteins, in addition to myoglobin and creatine kinase, appear in blood when amphetamine produces pronounced hyperthermia. Thus, we speculate that some vascular damage could be produced in the MAV, choroid plexus and the remaining brain vasculature as a result of proteins released by muscle during neurotoxic exposures to amphetamines. The exact mechanism by which this vascular damage may occur through muscle-derived serum proteins has yet to be explored.

Neurotoxic doses of METH can produce liver necrosis and elevate blood levels of ammonia.[108] However, this is very likely due to hyperthermia since very high doses have been reported not to produce histopathology when conducted under normothermic conditions. When histopathology and adverse liver enzyme changes present in blood serum are compared, EIH and neurotoxic exposures to AMPH have similar adverse effects.[103,109] Furthermore, liver necrosis is not necessary for either dopamine terminal damage or neurodegeneration but lesser perturbations in liver function still may exacerbate such processes.[103] Finally, although AMPH can significantly elevate liver-specific alanine transaminase, and ammonia, in some strains of Sprague-Dawley rats,[108] it minimally elevates this enzyme in other strains when AMPH produces neurotoxicity.[103] One mechanism by which AMPH disruption of liver function may influence neurotoxicity is through liver glycogen depletion.[103] This glycogen depletion may be behind the low blood glucose levels (70 to 40 mg/ dL) that normally occur as a result of neurotoxic exposures to AMPH and always precede body tremors and death (levels lower than 25 mg/ dL) (Bowyer, unpublished data).

Amphetamine and Hyperthermia Activation of the Circulating Immune System and Neurotoxicity

Neuroinflammation is a primary factor in neurological diseases such as multiple sclerosis.[110-113] Neuroimmune system dysfunction more recently has also been implicated to varying extents in the pathogenesis of Alzheimer's as well as being a hindrance to treating the disease.[114-117] Also, neuroinflammation has been implicated in Parkinson's disease.[118-120] Neuroinflammation (microglial activation and astrocytosis) is known to occur in brain regions where terminal damage and neurodegeneration is found when METH and/ or AMPH produce pronounced hyperthermia.[18,54,121-126] On the other hand, hyperthermia alone (EIH) produces minimal or no neuroinflammation in these brain regions. The importance of these effects in humans has been exemplified by the exacerbation of HIV neuropathology and drug addiction.[127-129] However, the research to date has not supported that neuroinflammation exacerbates the neurotoxicity to dopaminergic terminals produced by amphetamines but that neuroinflammation instead results secondarily to neurotoxicity.[123,130] Little research information is available as to whether neuroinflammation exacerbates the neurodegeneration seen in the various brain regions produced by AMPH or METH.

Neurotoxic exposures to AMPH produce greater immune responses in the MAV than other brain regions and choroid plexus.[43,93] Hyperthermia has a significantly lesser effect on the expression of genes related to inflammation in MAV. Somewhat

unexpectedly, the immune response in the choroid plexus to AMPH was much less than the MAV and was not that different from the immune response produced by EIH. In the case of the MAV, AMPH produces increases in the transcripts for the cell determinant protein *Cd14* and *Lbp*, which are genes relatively specific for microglia.[43] It is not yet known whether this translates into an increase in the number of macrophages present or an increased expression within individual macrophages. Additionally, it is not known whether the changes are occurring in the unique macrophages resident on the meningeal surfaces or are circulatory macrophages adhering to the endothelial cells in the lumen in damage areas of the vasculature in the MAV. It remains to be determined whether or not the increased immune response in MAV evoked by AMPH influences neurodegeneration within the underlying cortex.

Less is known about how neurotoxic exposures to amphetamines and hyperthermia affect the immune response tissues outside the brain and whether these changes influence the neurotoxic effects of amphetamines but information is emerging.[131] Initial published results and ongoing studies indicate that the significant immune responses in the circulating blood evoked by neurotoxic exposures to AMPH and hyperthermia alone (EIH) are often pronounced just prior to the onset of neurotoxicity to at least 1 day after exposure[103] (elevated protein levels IL-6, IL-10; and mRNA for *IL-1b, Cd8a, Cxcr2, Itgam,* and *Tnfrsf1a* unpublished data in GEO, NCBI; data file GSE29733). Tumor necrosis factor α levels were elevated 2-fold compared to control 1 day post AMPH. AMPH was observed to produce significantly higher levels of myoglobin in the serum than EIH indicating a greater damage to muscle. The release of proteins from muscle could well serve as damage associated molecular proteins (DAMPs) and activate the immune system.[132,133] DAMPs appear to play an important role in the inflammation process in pancreatitis.[134] It is interesting to note that AMPH, but not EIH, can trigger a tremendous increase in the expression of mRNA (*Reg3a* and *Reg3b,* biomarkers for pancreatic inflammation) in the MAV (but not blood, cortex, striatum or choroid plexus).[43] It is not known how the cell types expressing these genes are affecting meningeal function and whether it exacerbates cortical neurodegeneration.

Activation of the immune system could also occur through damage to the liver which can be produced by hyperthermia (EIH) as well as AMPH.[103] Regardless of how the immune response evoked by amphetamines in the circulating blood occurs, almost nothing is known as to how this alters the neurotoxic effect in the brain. There are reports that activating the immune system with LPS can exacerbate damage to dopaminergic systems by neurotoxins but this does not appear always to be the case with amphetamines.[135] It is not known whether this is the case for other regions of the brain, such as parietal cortex, thalamus and hippocampus, where amphetamines can produce neurodegeneration. Finally, very little is known as to whether activation of the immune response in circulating blood and the periphery plays a part in the transient psychosis that can occur with the abuse or prolonged use of amphetamines.[136-138]

Summary

In animal models that evaluate the neurotoxicity of AMPH and METH, it is quite clear that hyperthermia is one of the essential components necessary for the production of histological signs of dopamine terminal damage and neurodegeneration in cortex, striatum, thalamus and hippocampus. When animals remain normothermic during AMPH or METH exposure, only transient depletions of striatal dopamine occur (1 month or less) along with rare sporadic occurrences of neurodegeneration in the parietal and piriform cortex. The dopamine terminal damage and neurodegeneration that occur when amphetamines produce hyperthermia are likely due, in part, directly to hyperthermia increasing ROS, protein misfolding/ dysfunction and altering ion channel permeability in the affected neurons. Hyperthermia can also indirectly enhance neurodegeneration produced by amphetamines through the triggering of repetitive seizure activity. The generation of repetitive seizures and status epilepticus that can be produced by AMPH or METH is likely due to a breakdown in the BBB in the amygdala and hippocampus. Growing information also implicates that hyperthermia during exposure to amphetamines may affect the neurodegeneration produced indirectly through MAV dysfunction (vasospasm and ischemia) and damage to the choroid plexus (adverse effects on CSF function). Finally, it is possible that muscle and liver damage that are exacerbated by hyperthermia play significant roles in the neurotoxicity of amphetamines through releasing cellular proteins and other toxic substances into the circulation and/ or activation of the systemic immune system which might subsequently exacerbate neuroinflammation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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