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Breakdown of Blood-Brain and Blood-Spinal Cord Barriers During Acute Methamphetamine Intoxication: Role of Brain Temperature

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Abstract

Methamphetamine (METH) is a powerful and often abused stimulant with potent addictive and neurotoxic properties. While it is generally believed that structural brain damage induced by METH results from oxidative stress, in this work we present data suggesting robust disruption of blood-brain and blood-spinal cord barriers (BBB and BSCB) during acute METH intoxication in rats. We demonstrate the relationships between METH-induced brain hyperthermia and widespread but structure-specific barrier leakage, acute glial activation, changes in brain water and ionic homeostasis, and structural damage of different types of cells in the brain and spinal cord. Therefore, METH-induced leakage of the BBB and BSCB is a significant contributor to different types of functional and structural brain abnormalities that determine acute toxicity of this drug and possibly neurotoxicity during its chronic use.

Keywords

psychomotor stimulants; methamphetamine; brain hyperthermia; skin vasoconstriction; neurotoxicity; brain edema; cellular damage

Introduction

Methamphetamine (METH) is a powerful and often abused psychomotor stimulant with potent addictive and neurotoxic properties. Considering the issue of neurotoxicity, it is usually assumed that METH has direct toxic effects on neural cells, with relative selectivity towards specific cell groups, brain structures, and cellular organelles. Particularly, METH preferentially affects midbrain dopamine (DA) cells, damaging fine axonal terminals in the striatum [1–3] and resulting in health complications associated with pathologically altered DA transmission. Particularly, perturbations in DA as well as other monoamine systems are

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CONFLICT OF INTEREST

The authors report no conflict of interests

an important factor in psycho-emotional and movement disorders including acute METH psychosis and severe depression following long-term METH use and withdrawal [4].

However, METH also induces metabolic activation [5,6]. Enhanced metabolism is tightly related to oxidative stress, which is caused by an imbalance between the production of reactive oxygen and a biological system's ability to detoxify readily the reactive intermediates or easily repair the resulting damage. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress as a consequence of brain hyper-metabolism is usually viewed as a primary factor in METH-induced neurotoxicity [7–10].

It is also known that METH dose-dependently increases brain and body temperatures [11–14] and this hyperthermic effect is usually viewed as a consequence of drug-induced metabolic activation. Similar to other psychomotor stimulant drugs, METH also induces a powerful peripheral and central vasoconstriction [15,16] that prevents proper dissipation of metabolic heat from the brain and to the external environment, thus also contributing to heat accumulation and hyperthermia. Since brain cells are exceptionally temperature-sensitive, with the appearance of irreversible changes in structure and functions at ~40°C, i.e., only three degrees above normal baseline [17–20], high temperature *per se* could be a factor inducing damage of brain cells. Due to the strong temperature-dependence of most physico-chemical processes governing neural activity, brain hyperthermia could also strongly modulate toxic effects of METH on brain cells. It is well known that METH is much more toxic at high environmental temperatures, while toxicity is diminished by low ambient temperatures [11,14,21–24].

In addition to the direct effects on brain cells and potentiation of toxic effects of metabolic products, brain hyperthermia could also affect the permeability of the blood-brain and possibly blood-spinal cord barriers (BBB and BSCB). These barriers protect the brain environment, while leakage of these barriers allows water, ions, and various potentially neurotoxic substances contained in blood plasma to enter the brain [25,26]. Although leakage of the BBB has been documented during environmental warming [27,28], intense physical exercise [29], various types of stress [30–32] and morphine withdrawal [33], in this work we present data suggesting profound leakage of the BBB and BSCB during acute METH intoxication. We also consider the role of brain hyperthermia in the development of METH-induced barrier leakage as well as subsequent changes in glial activity and structural damage of brain and spinal cord cells. While the original data for BBB have been published [34,35], these data were combined with more recent data on BSCB [36], thus allowing us to consider common changes and differences in neural response to METH occurring in these two distinct but inseparable and equally important parts of the nervous system.

METH intoxication results in BBB and BSCB leakage, acute glial activation and morphological alterations of brain cells: role of brain temperature

Similar to our previous experiments, rats were implanted with three thermocouple electrodes (nucleus accumbens or NAcc, temporal muscle, and skin) and an intravenous (iv) jugular

catheter, habituated to the experimental conditions, and divided in three groups. Rats from the first two groups received METH (9 mg/kg, sc) under standard (23°C) and warm (29°C) ambient temperatures, respectively. As shown previously [14], warm ambient temperature strongly enhances hyperthermic effects of METH. When brain temperature peaked or reached clearly pathological values ($>41.5^{\circ}\text{C}$), the rats were injected with Evans blue (EB), rapidly iv anesthetized with pentobarbital, perfused, and their brains were taken for analysis. Control animals received saline and underwent the same procedures as METH-treated rats. The state of BBB permeability and edema were determined by diffusion into brain tissue of EB dye, an exogenous tracer that is normally retained by the BBB, and measuring brain water and ion (Na^+ , K^+ , Cl^-) content. Immunohistochemistry was used to evaluate quantitatively brain presence of albumin, a measure of breakdown of the BBB, and glial fibrillary acidic protein (GFAP), an index of astrocytic activation. Albumin is a relatively large plasma protein (molecular weight 59 kDa, molecular diameter 70A) that is normally confined to the luminal side of the endothelial cells and is not present in the brain. Similarly, EB, the exogenous protein tracer that binds to plasma albumin, fails to enter the brain from the peripheral circulation [37,38]. Thus, the presence of EB in the brain tissue and the appearance of albumin-positive cells and albumin immunoreactivity in the neuropil indicate a breakdown of the BBB. GFAP is an intermediate filament protein that is expressed in glial cells (astrocytes). Increased GFAP immunoreactivity (or astrocytic activation) is usually viewed as an index of gliosis or a relatively slow-developing correlate of neural damage [39,40]. Normal brain tissue has only scattered GFAP-positive cells but rapid GFAP expression has been reported previously during environmental warming and brain trauma [28, 41].

To determine morphological cell abnormalities, slices of brain and spinal cord tissue were stained with haematoxylin-eosin or Nissl and analyzed with light microscopy to determine the extent and specifics of structurally abnormal cells. For more detailed analyses of cellular and subcellular alterations, we also used transmission electronic microscopy (TEM). To determine the specificity of METH-induced changes in brain parameters, they were analyzed separately in the cortex, hippocampus, thalamus, and hypothalamus and in the spinal cord.

Alterations in the BBB permeability

As shown in Fig. 1A, METH induced significant increases in brain and temporal muscle temperatures at both ambient temperatures and these increases were significantly larger when the drug was administered at 29°C (NAcc: $41.37 \pm 0.22^{\circ}\text{C}$; muscle: $40.44 \pm 0.19^{\circ}\text{C}$) compared to 23°C (NAcc: $38.92 \pm 0.34^{\circ}\text{C}$; muscle: $37.92 \pm 0.32^{\circ}\text{C}$). METH also induced significant and widespread leakage in both BBB and BSCB. Compared to saline-treated controls, both EB staining (Fig. 1C) and albumin immunoreactivity (Fig. 1E) increased strongly in each METH group and the changes were significantly larger when the drug was administered at 29° than 23°C. Temperatures in both locations were tightly correlated ($r=0.98$) and temperatures in the NAcc were always larger than those in the temporal muscle in each rat of both groups (Fig. 1B).

Figure 2 shows profound differences in EB staining in brain and spinal cord tissues found in rats administered with METH at standard and warm ambient temperatures. While in control

rats blue staining was virtually absent in both the brain and spinal cord (a, d), superficial and paraventricular areas of the brain (b) as well as dorsal and ventral horns of the spinal cord (e) were blue stained in rats after METH exposure at 23°C. This staining amplified and greatly extended, involving most brain structures (c) and larger areas of horn tissue in rats exposed to METH at 29°C. While mean changes in EB concentrations were similar in both brain and spinal cord tissue, all values, including those in controls, were slightly larger in the brain (Fig. 1C).

Albumin immunoreactivity showed similar increases when METH was used at different temperatures and changes in the brain were stronger than those in spinal cord (Fig. 1E). As shown in representative examples of cortical and spinal cord tissue (see Fig. 3 and 4, top panels), the number of albumin-stained cells and intensity of staining in both structures were clearly larger after METH exposure and these changes further increased when the drug was administered at 29°C.

Both parameters reflecting barrier permeability: EB staining and albumin immunoreactivity, were tightly related to brain temperature (Fig. 1D and F). Both in the brain and spinal cord, EB levels and numbers of albumin-positive cells were minimal at low temperatures (control) and progressively increased in drug-injected animals. This correlation was linear and equally strong in both the brain and spinal cord.

Glial changes

METH treatment strongly increased the number of GFAP-positive astrocytes in both the brain and spinal cord (Fig. 5A and 6). The changes were almost identical, with a slightly larger response in the brain. Changes in GFAP immunoreactivity were tightly and linearly correlated with content of EB (Fig. 5B) and albumin immunoreactivity (Fig. 5C), suggesting close relationships between leakage of the BBB and acute glial reaction. GFAP counts were also tightly correlated with brain temperatures (Fig. 5D), suggesting that acute glial reaction is progressively larger depending on the extent of brain temperature elevation. Similar to EB and albumin, changes in the brain and spinal cord were very similar, coefficients of correlations were equally strong, and regression lines for both structures were almost superimposable.

Alterations in water and ion content

The ability of brain to maintain highly stable ionic and water homeostasis is essential for maintaining normal physiological and behavioral functions and even relatively small changes in ion and water content in brain tissue result in dramatic impairments in brain activity and functions. Tissue water accumulation (vasogenic edema) is a serious, life-threatening condition that could result in organism's death. In agreement with the previous findings on regional differences in brain water content [25], we found that spinal cord tissue has ~12% less water than brain tissue (Fig. 7). While in both the brain and spinal cord water content increased during METH treatment and became stronger when the drug was administered at 29°C, the changes were quantitatively stronger in the spinal cord, with about 3% difference between control and METH-29°C groups (A and B). Interestingly, in control conditions different brain structures have different water content, with the highest values in

the hippocampus (78.4%) and lower values in the cortex (74.5%) and the underlying thalamus (75.5%) and hypothalamus (75.5%). The cortex and hippocampus also exhibited larger increases in water content after METH treatment (29°C: ~2.9 and 3.1%, respectively) than the thalamus and hypothalamus (2.8 and 2.6%, respectively).

Comparison of ionic response seen in the spinal cord and the brain revealed a general similarity in changes of Na^+ , Cl^- , and K^+ concentrations (Fig. 7C and D). Both Na^+ and Cl^- increased during METH intoxication, and the increase was larger at 29°C. Changes in K^+ were weaker, but both in spinal cord and the brain concentration of K^+ significantly increased in METH-29°C group. Basal levels of all ions did not differ between the spinal cord and the brain. By using regression analysis, we show that water accumulation in both the brain and spinal cord tissue directly correlates with the barrier leakage (albumin-positive cells; Fig. 8A), glial activation (GFAP-positive cells; Fig. 8B) and the magnitude of METH-induced brain temperature elevation (Fig. 8C). Despite robust differences in water content between the brain and spinal cord, within the entire animal sample, correlation was equally strong for each pair of parameters.

Structural changes

As shown in Fig. 9, METH treatment resulted in a profound increase in the amount of abnormal neural cells in each studied brain structure and in the spinal cord. In rats that received METH at standard ambient temperature, the numbers were lower than in rats that received METH at warm ambient temperature and showed extreme hyperthermia. The increase vs. control (where abnormal cells were virtually absent) was significant in each region but in the METH-23°C group the highest numbers were seen in the hippocampus followed by the thalamus, hypothalamus, and cortex. In terms of relative change, the hippocampus also showed the maximal effect (x52), followed by hypothalamus (x30), cortex (x21), and thalamus (x20). The incidence of neuronal damage was almost 1.5 times greater in animals that received METH at 29°C. In this case, the greatest changes were seen in the cortex, followed by the hippocampus, thalamus, and hypothalamus. Interestingly, spinal cord cells assessed under identical conditions were less prone to damage, showing minimal values in both drug-treated groups.

Representative examples of Nissl-stained sections showing nerve cell damage in the parietal cortex and spinal cord are depicted in Figs. 3 and 4. Control animals in both brain areas exhibit healthy neurons with a distinct nucleus and clear cytoplasm, while several pyknotic neurons (arrows), perineuronal edema, and tissue sponginess were seen in METH-treated rats. These changes were clearly more pronounced in rats that received METH at 29°C and showed extreme hyperthermia. More details on drug-induced structural changes are shown on TEM images (Fig. 10).

Relationships between structural abnormalities of neural cells and other parameters

Changes in brain morphology were associated with profound changes in all other analyzed parameters (Fig. 11). Neural damage in both the brain and spinal cord during METH intoxication was tightly directly correlated with brain temperature increases (A), albumin leakage (B), acute glial activation (C), and rise in tissue water content. In the control

conditions, when temperatures were at their physiological baseline, albumin leakage and glial activation were virtually absent, there were no abnormal cells but their number grew progressively in METH-treated brains, depending on the extent of changes in brain parameters. For each parameter this correlation was strong, linear, and virtually equal in the brain and spinal cord, indicating its generality for the CNS as a whole. The number of damaged cells in both the brain and spinal cord during METH intoxication correlates linearly with water accumulation in all brain areas. This relationship appears to be a reflection of generalized edema, which progresses when METH is used at 29°C, resulting in more profound cellular damage.

While brain temperature was recorded in this study only from the NAcc, it should follow a dorso-ventral temperature gradient and be minimal in the cortex, higher in hippocampus and thalamus, and maximal in the hypothalamus [42]. However, the damage in ‘warm’ hypothalamus during METH intoxication was minimal but it was maximal in the ‘cool’ cortex. In contrast, structural damage was less evident in the spinal cord, where tissue temperature should be lower than that recorded from the NAcc, a ventrally located brain structure.

Functional implications

While slowly developing, selective, and irreversible damage of specific central neurons is a traditional focus of neurotoxic studies of METH, this work demonstrates that robust morphological abnormalities of neural and non-neural cells in the brain and spinal cord (i.e., glia, vascular endothelium, epithelium) could occur rapidly (within 30-80 min) during acute METH intoxication. These abnormalities manifest as the distortion of neurons, overexpression of glial cells, vesiculation of myelin, as well as alterations in vascular endothelium and epithelium of the choroid plexus. While having some structural specificity, these acute morphological abnormalities appear to be widespread, tightly correlating with drug-induced brain and body hyperthermia and alterations in several basic homeostatic brain parameters including permeability of the BBB, tissue water and ion contents.

Mechanisms underlying METH-induced morphological brain abnormalities

Our study revealed that acute morphological alterations of brain and spinal cord cells during METH intoxication are tightly related to drug-induced increases in brain and muscle temperatures. The tight link between hyperthermia and structural brain damage was evident for the different brain structures and for both neuronal (Nissl staining) and glial cells (GFAP immunoreactivity). Although METH was used in all animals at the same dose and a “pharmacological toxic impact” was identical in each case, the counts of damaged neurons and abnormal GFAP-positive glial cells were drastically larger at 29°C than 23°C, correlating with stronger brain and body hyperthermia. Linear relationships between these parameters and brain temperature were also evident in individual animals, which showed greater morphological abnormalities at higher temperatures, irrespective of the ambient temperature during drug administration.

Since brain cells are exceptionally sensitive to high temperature [18,19,20,43–46], hyperthermia could be viewed as an important contributor to morphological abnormalities

induced by METH. However, this does not mean that high temperature *per se* is the cause of these changes. Brain hyperthermia is not only a physical factor that could harm cells; it is also an integral physiological index of METH-induced metabolic activation [see 42 for review] that also manifests as an enhanced release of multiple neuroactive substances, lipid peroxidation, and the generation of free radicals—numerous changes combined as oxidative stress [7, 47] as well as behavioral and autonomic activation. Although all these factors may contribute to structural brain abnormalities, it is quite difficult to separate them from each other because they are interdependent, representing different manifestations of METH-induced metabolic activation.

METH intoxication also results in a robust increase in BBB and BSCB permeability, water accumulation in neural tissue (vasogenic edema), and serious alterations in brain ionic homeostasis. These changes, moreover, were tightly related to both the degree of hyperthermia and the intensity of structural brain damage. Therefore, breakdown of the BBB that allows the diffusion of endogenous albumin, water, several ions, and other neuroactive and potentially neurotoxic substances is another important contributor to brain pathology and the primary mechanism underlying decompensation of vital functions and possible lethality during METH overdose. While different chemical factors activated by METH could be involved in increased BBB permeability and edema formation, brain hyperthermia appears to play a crucial role since both these parameters strongly correlated with brain temperature. Such a role for hyperthermia in the leakage of the BBB and edema has been first suggested for environmental warming [27,28]; the intensity of these changes was strongly dependent upon the strength of body hyperthermia as evaluated by rectal temperature measurements.

Common features and structural selectivity of METH-induced cellular alterations

Despite a general commonality, METH-induced morphological and functional perturbations induced by METH had structural specificity. As shown above, individual brain structures and spinal cord had some differences in tested parameters in normal conditions as well as some degree of specificity following METH impact. Despite evident between-structure differences in tissue water content in control, the increase was relatively similar in different brain structures at 23°C (~0.8%) and dramatically amplified at 29°C (~2.5%), suggesting severe edema within the brain as a whole. While water content in the spinal cord was 10-12% lower, its levels increased much stronger during METH intoxication (~1.8 and ~3.2% in METH-23°C and METH-29°C groups). Robust water accumulation in brain tissue was associated with a clearly pathological brain hyperthermia (>41°C), strong increases in Na⁺, K⁺, Cl⁻ in all brain structures, and maximal increases in albumin- (x22-75) and GFAP-positive (x8-13) cells. These animals also showed especially strong cell abnormalities that were evident in each structure. While edema and ionic misbalance appears to be widespread within the brain, individual brain structures differed in the extent of morphological abnormalities. The numbers of damaged neuronal and glial cells were both greater in the cortex and hippocampus but smaller in the thalamus and hypothalamus. While the reasons of this structural specificity to damage remains unknown, the cortex and hippocampus also showed much stronger increases in EB penetration and albumin immunostaining than the

thalamus and hypothalamus, suggesting a tight link between BBB leakage and structural damage.

Reversibility/Irreversibility of structural alterations

Although our data indicate that acute METH intoxication results in rapidly developing structural abnormalities in neural and non-neural cells in the brain and spinal cord, it remains unclear whether these abnormalities are reversible or irreversible in nature. Dramatic changes in cellular elements (e.g., degeneration of some neuronal nucleus, endothelial cell membrane bleb-formation, vesiculation of myelin and vacuolation in the neuropil with clear signs of synaptic damage and swelling), especially evident in the METH-29°C group, appear to be inconsistent with normal cell functions, pointing at irreversible damage. However, some of these changes appear to be transient and reversible and they could dissipate after basic homeostatic parameters are restored to baseline. In contrast to the unusually large numbers of GFAP-positive glial cells found in this study during acute METH intoxication (x3-7 and x7-12 vs. control in 23 and 29°C groups, respectively), much weaker increases in GFAP-positive cells were found in rats in days after acute METH impact [48,49]. These changes, moreover, appeared at 12-24 hrs, peaked at the second day, and remained elevated for seven days after a single METH exposure. In contrast to the widespread alterations in our study, these changes were evident only in the striatum and to a lesser degree in the cortex, correlating with decreased dopamine levels in these structures. While in light of the profound morphological abnormalities found during acute METH intoxication we can speculate that they could result in irreversible cellular damage, this issue needs to be examined further. While rapid and strong increases in GFAP immunostaining have been reported previously during environmental warming (~2-3 hours [27,28]) and following acute traumatic injury to the brain and spinal cord (30-40 min [28,41]), different mechanisms appear to mediate rapid and slow glial reactions. GFAP expression is usually thought of as a late outcome of traumatic, ischemic, or hypoxic insults or a correlate of various neurodegenerative diseases [39–41], representing astrogliosis [50,51]. In contrast, rapid GFAP expression seen in association with strong edema (environmental warming, acute trauma, METH intoxication) could reflect the interaction of antibodies with GFAP somehow released or made available during membrane damage. Thus, binding sites to GFAP could be increased due to acute breakdown of the BBB and associated edema rather than proliferation of astrocytes or elevated levels of GFAP proteins that require more time. Since damage of astrocytes and swelling of the astrocytic end foot results in increased binding of GFAP antibodies [41,52,53], this reaction could reflect acute, possibly reversible, damage of glial cells. Relatively smaller numbers of abnormal neural cells in the post-intoxication period compared to acute METH intoxication [54] could also be related to their rapid scavenging, making it difficult to detect them using traditional techniques. Although the issue of the extent of damage and its reversibility remains unanswered and requires additional studies, it is likely that rapid cell abnormalities may initiate cascades that could precipitate cellular and molecular dysfunctions, leading to neurodegeneration—the most dangerous outcome of chronic abuse with amphetamine-like drugs.

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LIST OF ABBREVIATIONS

BBB	blood-brain barrier
BSCB	blood-spinal cord barrier
EB	Evans blue
GFAP	glial fibrillary acidic protein
iv	intravenous
METH	methamphetamine
NAcc	nucleus accumbens
sc	subcutaneous

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Fig. 1. Effects of methamphetamine (9 mg/kg) on brain (NAcc) and muscle temperatures (A), concentrations of Evans blue (B) and albumin immunoreactivity (C) in the brain and spinal cord rats during acute METH intoxication at 23 and 29°C. Asterisks show values significantly larger than control and small circles show significant values between two METH groups. B shows the relationships between NAcc and muscle temperature and D-F show relationships between NAcc temperature and two indices of barrier permeability: Evans blue (D) and Albumin (F). Each graph shows two regression lines and two coefficients of correlation for spinal cord and brain, respectively.

Fig. 2. Schematic diagram of coronal sections of the brain passing through hippocampus (a-c; bregma -4.30 mm) and spinal cord T9 (d-f) showing visual distribution of Evans blue dye as seen by gross examination under a magnifying lens (x4) in control (a, d) and after METH exposure at 23 °C (b, e) or 29°C (c, f). Gross examination showed that leakage of EB was the most prominent and widespread in METH-treated rats at 29°C in both the brain and spinal cord sections as compared to 23°C. Bar = 5 mm. Spinal cord data modified after Kiyatkin and Sharma (2015).

Parietal Cerebral Cortex

Fig. 3.

Albumin immunoreactivity (a-c) and Nissl staining (d-f) in the parietal cerebral cortex in rats in control (a, d) and after METH administration at 23 (b, e) and 29°C (c-f). The leakage of albumin and perineuronal edema were more pronounced at 29°C compared to 23°C. The albumin immunoreaction was seen within the neuronal cytoplasm and in most cases karyoplasm (arrows). Some albumin-positive cells with small, rounded bodies and without any apparent cell nucleus appear to be glial cells. (b, c). Control animals exhibited a few albumin-positive cells, some of which could also be glial cells. In METH-treated rats Nissl staining shows several distorted and damaged neurons in identical areas of the parietal cerebral cortex that exhibited albumin immunoreactivity (e, f). The magnitude and intensity of neuronal damages was clearly larger in METH-29°C compared to METH-23°C groups. While control, saline-treated rats showed healthy neurons with clear cell nucleus (d), spongy neuropil associated with vacuolation and edema was found in METH-treated animals.

T9 Spinal cord

Fig. 4.

Albumin leakage (a-c) and Nissl staining (d-f) in spinal cord tissue in rats in control (a, d) and after METH administration at 23 (b, e) and 29°C (c-f). Similar to the brain, spinal cord also showed extensive leakage of albumin following METH treatment at 29°C as compared to 23°C. Also, neuronal damages included neuronal shrinkages (arrows), perineuronal edema and neuronal distortions, which were most prominent in METH-treated rats at 29°C. Control rats showed healthy neurons (d) and only few albumin-positive cells that look like astrocytes (a).

Fig. 5.

Mean ($\pm$ sem) numbers of GFAP-positive cells in the brain and spinal cord in rats injected with saline (control) and METH at 23°C and 29°C (A). B shows correlative relationships between the number of GFAP-positive cells and Evans blue content in neural tissue of the brain and spinal cord. C shows correlative relationships between the numbers of albumin- and GFAP-positive cells in the brain and spinal cord. D shows correlative relationships between the numbers of GFAP-positive cells in the brain and spinal cord. Each graph shows regression lines and correlation coefficients (r).

T9 Spinal cord

Parietal Cerebral Cortex

Fig. 6.

Reactive astrocytes in the parietal cortex (d-f) and T9 segment of the spinal cord (a-c) as demonstrated by GFAP immunostaining in control and METH-treated rats that received the drug at 23°C (b, e) and 29°C (c, f). GFAP immunoreactivity in both the brain and spinal cord was much stronger and widespread when METH was given at 29 compared to 23°C. Control rats showed only scarce GFAP immunoreaction around the microvessels (a, d) and possibly in some dendrites. GFAP immunoreactivity was especially strong around neurons and microvessels; reactive astrocytes was also seen in the neuropil (arrows).

Fig. 7.

METH intoxication results in alterations of ionic homeostasis and water accumulation in spinal cord and brain tissue. Left panel shows mean ($\pm$ sem) changes in tissue water (A, %) and ions (B, Na⁺, K⁺, and Cl⁻, mM/kg) in the spinal cord in animals of each group.

Asterisks show significance vs. control and small circles show significance between two METH groups. Right panel shows changes in the same parameters determined in the brain.

Fig. 8.

The relationships between tissue water content and albumin immunoreactivity (A), GFAP immunoreactivity (B), and brain temperature (C) shown separately for the brain and spinal cord. Each graph shows regression line and correlation coefficient (r).

Fig. 9.

Mean ($\pm$ sem) numbers of structurally abnormal cells in individual brain structures and spinal cord after saline (control) and METH administration at 23°C and 29°C.

T9 Spinal cord

Parietal Cerebral Cortex

Control

Meth 23°C

Meth 29°C

Fig. 10.

Low-power transmission electron micrographs from spinal cord (a-c) and parietal cerebral cortex (d-f) in control (a, d) and METH-treated rats that received the drug at 23 (b, e) and 29 °C (c, f). In the spinal cord myelin damage and vesiculation were most prominent after METH treatment at 29 °C (c) as compared to 23°C (b, arrows). In the brain, microvascular reactions such as perivascular edema, endothelial cell distortion or blebs (e, f; arrows) were very common in METH-treated group (e, f). In METH-treated rats vacuolation and water-filled cells could be also seen in the neuropil (arrows) and these changes were stronger in rats that received the drug at 29°C (f) compared to 23°C (e).

Fig. 11.

METH-induced cellular damage in both the spinal cord and brain depends on brain temperature elevation (A), the extent of albumin and GFAP immunoreactivity (B and C, respectively) and water tissue accumulation (D). Each graph shows two coefficients of correlation (r) for the brain and spinal cord data samples. All coefficients of correlation were highly significant ($p<0.001$).