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Short communication

Phenobarbital and dizocilpine can block methamphetamine-induced neurotoxicity in mice by mechanisms that are independent of thermoregulation

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Abstract

Body temperature profiles observed during methamphetamine (METH) exposure are known to affect dopamine and tyrosine hydroxylase (TH) levels in the striatum of mice; hyperthermia potentiates depletion while hypothermia is protective against depletions. In the current study, the doses of METH were sufficiently great that significant dopamine and TH depletions occurred even though hypothermia occurred. Four doses, administered at 2 h intervals, of 15 mg/kg (4×15 mg/kg) D-METH significantly decreased TH and dopamine levels to 50% of control in mice becoming hypothermic during dosing in a 13°C environment. Phenobarbital or dizocilpine during METH exposure blocked the depletions while diazepam did not. Phenobarbital and dizocilpine did not block depletions by altering the hypothermic profiles from that observed during METH only exposure. Here we show that phenobarbital and dizocilpine can block measures of METH neurotoxicity by non-thermoregulatory mechanisms.

Theme: Neurotransmitters, modulators, transporters and receptors

Topic: Catecholamines

Keywords: Methamphetamine; Hypothermia; Striatum; Dopamine; Neurotoxicity

In both rat and mouse, hyperthermia potentiates the dopamine and TH depletions and astrocytosis produced by METH or amphetamine (AMPH) exposure while hypothermia can protect against these effects [3–5,12,15]. In addition, compounds that protect against METH- or AMPH-induced dopamine depletions, such as haloperidol and dizocilpine, primarily have been ascribed to a blockade of hyperthermia resulting from administration of these compounds at room temperature [1,3,12]. It has also been observed that mice given high doses of METH are not always protected completely against dopamine depletions by a cold environment [2]. However, it was not determined in those experiments whether hyperthermia occurred even in the cold environment with the high doses of METH.

The results described here are based on the use of a dosing regimen administered at a reduced environmental temperature. This paradigm was used to evaluate the effects of pharmacological probes on METH neurotoxicity. The action of these same probes has been attributed to an effect on body temperature independent of their presumed mechanism of neuroprotection. By administering these compounds at a reduced ambient temperature, a clearer interpretation of how these agents may alter METH neurotoxicity can be obtained. Mice were administered 4×15 mg/kg doses of D-METH at 13°C as a potential dosing paradigm that would fit these criteria. It was also determined whether NMDA antagonists (dizocilpine), anticonvulsants (phenobarbital) and GABA modulators such as diazepam have protective affects against dopamine and TH depletions without altering the temperature profile produced by METH alone.

Abbreviations: AMPH, D-amphetamine; NMDA, *N*-methyl-D-aspartate; METH, D-methamphetamine; TH, tyrosine hydroxylase

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Prior to testing, 4 to 5-month-old male C57BL/6J mice were obtained from the breeding colony at NCTR/FDA (Jefferson, AR). They were housed with food and water available ad libitum, and were on a daily 12 h light cycle, with lights on at 6.00 a.m. Temperature (23±1°C) and humidity (53±15%) were controlled. During testing mice were housed three per cage to facilitate behavioral observations. One group of mice was dosed in a 23°C environment while the remaining groups were placed in a 13°C environment 30 min prior to METH exposure and remained there until up to 4 h after the last dose of METH. Four doses of 15 mg/kg (4×15 mg/kg) METH or 4×1 ml/kg saline s.c. were given at 2 h intervals. Groups of mice receiving METH at 13°C were also given two doses of 1 ml/kg normal saline (0.9% NaCl), 40 mg/kg phenobarbital, 0.5 mg/kg dizocilpine (MK-801) or 5 mg/kg diazepam administered i.p. The first dose given 15 min prior to the first dose of METH and the second dose given 15 min prior to the third dose of METH. Seven days after METH exposure the mice were sacrificed and striatum dissected on ice and stored at −150°C for later analysis of dopamine, TH levels.

Body temperatures were monitored as described by Bowyer et al. [3] except that a much smaller thermistor (model no. 520 from YSI Inc., Yellow Springs, OH 45387, USA) probe of about 1 mm in diameter was used to determine rectal temperatures. The body temperature of all animals was monitored starting from just prior to the first dose of METH. Temperatures were taken 1 h after every dose of METH and at 3 h after the last dose of METH. Previous and recent results from experiments in our laboratories indicates that temperatures determined at 1 h post-injection of METH are representative of the peak changes in temperature. In addition, those animals dosed in the cool environment with body temperatures above 35°C at 3 h after the last dose of METH were monitored for an additional hour. Animals whose temperature dropped below 30°C were placed in a 23°C environment, and were not returned to the 13°C environment until their temperature was above 32°C.

For each animal one striatum was used for determining dopamine, serotonin (5-HT) and their metabolites. The other striatum was used to determine TH levels. For striatal levels of aromatic monoamines and metabolites, each striatum was weighed and diluted with a measured volume (20% w/v) of 0.2 M perchloric acid. After sonication and centrifugation, the supernatant was removed and injected directly onto the high-performance liquid chromatography/electrochemical detection (HPLC/EC) system. The HPLC-quantitation of neurotransmitter and metabolites extracted from striata were similar to those of Stephans et al. [20]. The HPLC retention times were 3.25 min (DOPAC), 4.7 min (dopamine), 5.95 min (5-HIAA), 10.4 min (HVA), 13.1 min (5-HT) and 15.4 min (3-methoxytyramine). TH (holoenzyme protein) was assayed according to a modification of the detergent-based sandwich enzyme-linked immunosorbent assay (ELISA) of O'Callaghan [14] and Reinhard and O'Callaghan [16]. Briefly, a mouse monoclonal antibody to TH and a rabbit polyclonal antibody to TH (Calbiochem-Novabiochem Corp., Lavolla, CA, USA) were used to 'sandwich' TH present in detergent homogenates of brain regions. Quantification was achieved with a fluorescent substrate (Quantablu, Pierce Chemical Co., Rockford, IL, USA) generated by an HRP-conjugated secondary antibody using a Fmax plate reader (Molecular Devices Corp., Sunnyvale, CA, USA) set at 320/405 nm. TH levels were expressed in Fig. 1 as μg/mg of protein. Protein levels were determined by the methods of Smith et al. [18].

Data are presented as arithmetic mean±S.E.M. unless otherwise indicated. Multiple groups were analyzed by either a one-way analysis of variance (ANOVA) (Fig. 1) or a repeated measures two-way ANOVA (Table 1). A post hoc Tukey's least significant difference test was applied if significant main effects were observed.

The effects of METH and saline or METH in combination with phenobarbital, dizocilpine or diazepam on mouse body temperature can be seen in Table 1. The group given METH and saline in a 23°C environment had significantly higher body temperatures, over all time-points, than the other groups ($P<0.01$) except the saline and saline controls (not quite significant, $P=0.07$). Over the entire dosing period, the animals given METH and saline in a 13°C environment had significantly lower body temperatures than the METH and saline at 23°C but they did not differ from the saline and saline (13°C) controls or the METH plus phenobarbital group. The METH with diazepam and METH with dizocilpine had significantly lower body temperatures compared to the controls over the entire time-period ($P<0.01$ for both groups). Four of the 10 mice given METH and saline at 13°C had to be placed outside in a 23°C environment due to hypothermia (<30°C) while four of the 13 phenobarbital and METH, six of the 13 dizocilpine and METH and four of the 10 diazepam and METH mice were placed outside the cold environment. None of the groups dosed with METH at 13°C had significantly different body temperatures over the four doses.

The effect of METH and saline or in combination with other drugs on TH and dopamine levels can be seen in Fig. 1. METH and saline given in a 23°C environment reduced dopamine and TH levels significantly more than METH and saline at 13°C, however, the levels were reduced to 50% control even in the cooler environment. Both phenobarbital and dizocilpine significantly blocked the METH decreases in TH and dopamine observed at 13°C and their groups' levels were not significantly less than control. Diazepam did not block METH decreases in TH and dopamine although the degree of hypothermia it produced was as great as dizocilpine and greater than phenobarbital. Serotonin and 5-hydroxyindolamine levels in the striatum did not significantly differ among the groups and ranged

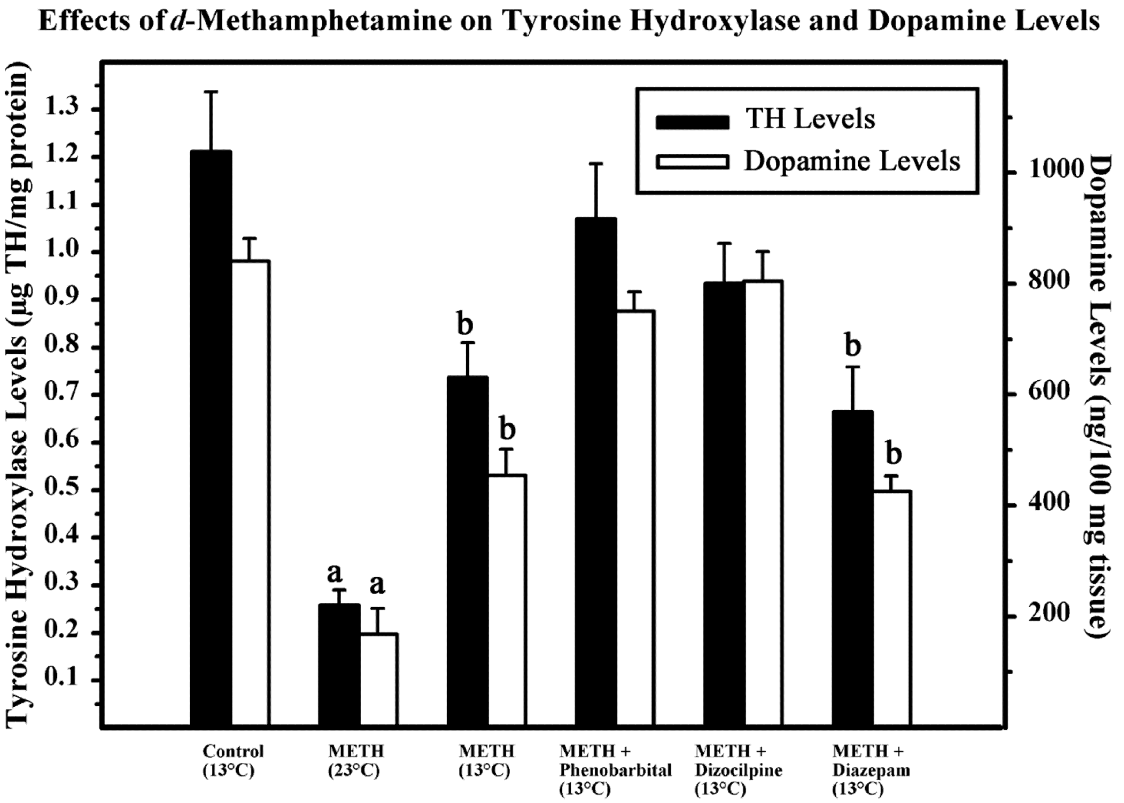

Fig. 1. Decreases in striatal dopamine and TH resulting from methamphetamine in combination with phenobarbital, dizocilpine or diazepam. The levels of TH and dopamine were determined 7 days post dosing. All groups were administered four consecutive doses, with 2 h between doses, of 15 mg/kg D-methamphetamine except the saline controls which were given four doses of 1 ml/kg saline ($n=9$). The groups dosed with METH in a 13°C environment were also given two doses of 1 ml/kg saline ($n=10$), 40 mg/kg phenobarbital ($n=13$), 5 mg/kg diazepam ($n=10$) or 0.5 mg/kg dizocilpine ($n=13$). There were five mice in the group receiving METH only at 23°C. [a]Indicates levels are significantly less than saline controls ($P<0.001$). [b]Indicates levels are significantly less than saline controls but greater than METH at 23°C ($P<0.05$).

Table 1
The effects of methamphetamine in combination with saline, phenobarbital, dizocilpine or diazepam on body temperature

Drug treatment	Body temperature at various time-points (in °C) during dosing					
	Start	1 h	3 h	5 h	7 h	9 h
Saline +saline	37.0±0.2°C	36.9±0.2°C	36.7±0.2°C	36.1±0.3°C	36.5±0.2°C	36.0±0.3°C
METH (23°C[a]) +saline	36.8±0.4°C	38.4±0.3°C	38.8±0.3°C	38.5±0.6°C	38.0±0.3°C	37.6±0.3°C
METH (13°C[a]) +saline	36.6±0.3°C	36.5±0.4°C	36.3±0.4°C	34.1±1.0°C	32.1±1.1°C	32.8±0.8°C
METH (13°C[a]) +phenobarbital	36.5±0.3°C	35.5±0.4°C	36.0±0.4°C	33.0±0.9°C	32.4±0.8°C	34.6±0.7°C
METH (13°C[a]) +dizocilpine	36.6±0.2°C	31.6±0.9°C	32.8±0.9°C	29.7±0.9°C	32.8±0.9°C	32.8±1.0°C
METH (13°C[a]) +diazepam	36.4±0.4°C	30.9±0.9°C	32.4±1.5°C	33.0±1.5°C	32.9±1.1°C	31.5±1.4°C

[a] Indicates the environmental temperature during dosing.

from 43.8±3.9 to 37.8±2.4 ng/100 mg tissue and 8.7±1.2 to 12.2±1.9 ng/100 mg tissue, respectively.

These studies indicate that in male C57Bl6/j mice, if the doses of METH are of sufficient magnitude, effects that are equated with neurotoxicity such as dopamine and TH depletions are seen even when hypothermia occurs during exposure. Until more extensive work is done, it will not be known whether exactly the same mechanisms are behind the METH-induced decreases in TH and dopamine when hypothermia occurs, compared to those produced in the presence of hyperthermia. Although one might assume this is a logical conclusion, it should be noted that in rat doses of up to 4×15 and 4×20 mg/kg do not produce dopamine depletions when hypothermia occurs during METH exposure [3,4]. Thus, it is not even certain that the phenomenon of high dose, temperature-independent, METH-induced dopamine and TH depletions extend throughout the rodent family.

It is possible that some of the effects of METH on TH and dopamine levels in mice are due to regulatory effects either on the synthesis of the enzyme TH or its activity, which are known to occur [11,23]. Changes in the rate of synthesis or turnover of TH could up- or down-regulate TH levels independent of terminal damage. In the rat, TH synthesis and levels in the substantia nigra appear unaltered after neurotoxic doses of AMPH but the levels in the striatum are significantly decreased possibly due to either breakdown/loss of TH during axonal transportation or increased turnover in the terminal [6,9,10]. TH synthesis in the substantia nigra can be altered in mouse after METH exposure [11]. Changes in the phosphorylation states of TH could affect dopamine levels independently of enzyme levels [11,23]. However, changes in the TH and dopamine levels after the various treatments correlate so closely that any changes in phosphorylation would have to be the same for all treatments.

There is no clear explanation as to why phenobarbital blocked TH and dopamine depletions produced by METH and diazepam did not. The reversal of METH and saline (13°C) induced TH and dopamine depletions produced by dizocilpine and phenobarbital could be due to their effects on TH synthesis. If this were the case, then these two drugs interact with METH in a way that affects the regulation of TH expression that benzodiazepines do not. This might not be expected since all three drugs are anticonvulsants and both diazepam and phenobarbital affect $GABA_A$ regulated chloride channels [17]. Anticonvulsants could affect repetitive neuronal firing [17] and perhaps through reducing glutamate release, affect damage to dopamine terminals [13,19,21,22]. Glutamatergic projections from both the somatosensory cortex [8] and the intralaminar nuclei of the thalamus [7] to the CPu could be responsible for the excessive release and increased CPu extracellular glutamate levels [13,21] that occur during METH exposure. However, again, one might expect diazepam to be as effective as dizocilpine or phenobarbital at blocking excessive neuronal firing/activity.

In conclusion, these results indicate that 4×15 mg/kg METH administered in a cold environment produces dopamine and TH depletions, and that mice dosed under these conditions become hypothermic. Furthermore, these dopamine and TH depletions can be reversed by phenobarbital and dizocilpine, drugs with anticonvulsant properties, without altering the hypothermic profile produced by METH. Thus, it seems likely that phenobarbital and diazepam can block METH neurotoxicity by mechanisms other than disrupting the thermoregulatory changes induced by METH.

References

[1] D.S. Albers, P.K. Sonsalla, Methamphetamine-induced hyperthermia and dopaminergic neurotoxicity in mice: pharmacological profile of protective and nonprotective agents, J. Pharmacol. Exp. Ther. 275 (1995) 1104–1114.

[2] S.F. Ali, G.D. Newport, R.R. Holson, W. Slikker Jr., J.F. Bowyer, Low environmental temperatures or pharmacologic agents that produce hypothermia decrease methamphetamine neurotoxicity in mice, Brain Res. 658 (1994) 33–38.

[3] J.F. Bowyer, D.L. Davies, L. Schmued, H.W. Broening, G.D. Newport, W. Slikker Jr., R.R. Holson, Further studies of the role of hyperthermia in methamphetamine neurotoxicity, J. Pharmacol. Exp. Ther. 268 (1994) 1571–1580.

[4] J.F. Bowyer, B. Gough, W. Slikker Jr., G.W. Lipe, G.D. Newport, R.R. Holson, Effects of a cold environment or age on methamphetamine-induced dopamine release in the caudate putamen of female rats, Pharmacol. Biochem. Behav. 44 (1993) 87–98.

[5] J.F. Bowyer, A.W. Tank, G.D. Newport, W. Slikker Jr., S.F. Ali, R.R. Holson, The influence of environmental temperature on the transient effects of methamphetamine on dopamine release in rat striatum, J. Pharmacol. Exp. Ther. 260 (1992) 817–824.

[6] J.F. Bowyer, L.T. Frame, P. Clausing, K. Nagamoto-Combs, C.A. Osterhout, C. Sterling, A.W. Tank, Long-term effects of amphetamine neurotoxicity on tyrosine hydroxylase mRNA and protein in aged rats, J. Pharmacol. Exp. Ther. 286 (1998) 1074–1085.

[7] J.F. Bowyer, S.l. Peterson, R.L. Rountree, J. Tor-Agbidye, G.J. Wang, Neuronal degeneration in rat forebrain resulting from D-amphetamine-induced convulsions is dependent on seizure severity and age, Brain Res. 809 (1998) 77–90.

[8] A.J. Eisch, J.F. Marshall, Methamphetamine neurotoxicity: dissociation of striatal dopamine terminal damage from parietal cortical cell body injury, Synapse 30 (1998) 433–445.

[9] H.C. Fibiger, E.G. McGeer, Effect of acute and chronic methamphetamine treatment on tyrosine hydroxylase activity in brain and adrenal medulla, Eur. J. Pharmacol. 16 (1971) 176–180.

[10] A.J. Hotchkiss, J.W. Gibb, Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain, J. Pharmacol. Exp. Ther. 214 (1980) 257–262.

[11] S.C. Kumer, K.E. Vrana, Intricate regulation of tyrosine hydroxylase activity and gene expression, J. Neurochem. 67 (1996) 443–462.

[12] D.B. Miller, J.P. O'Callaghan, Environment-, drug- and stress-induced alterations in body temperature affect the neurotoxicity of substituted amphetamines in the C57BL/6J mouse, J. Pharmacol. Exp. Ther. 270 (1994) 752–760.

[13] J.F. Nash, B.K. Yamamoto, Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4 methylenedioxymethamphetamine, Brain Res. 581 (1992) 237–243.

[14] J.P. O'Callaghan, Quantification of glial fibrillary acidic protein: comparison of slot-immunobinding assays with a novel sandwich ELISA, Neurotoxicol. Teratol. 13 (1991) 275–281.

[15] J.P. O'Callaghan, D.B. Miller, Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse, J. Pharmacol. Exp. Ther. 270 (1994) 741–751.
[16] J.F. Reinhard Jr., J.P. O'Callaghan, Measurement of tyrosine hydroxylase apoenzyme protein by enzyme-linked immunosorbent assay (ELISA): effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on striatal tyrosine hydroxylase activity and content, Anal. Biochem. 196 (1991) 296–301.
[17] M.A. Rogawski, R.J. Porter, Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage components, Pharmacol. Rev. 42 (1990) 223–286.
[18] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Measurement of protein using bicinchoninic acid, Anal. Biochem. 150 (1985) 76–85.
[19] P.K. Sonsalla, W.J. Nicklas, R.E. Heikkila, Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity, Science 243 (1989) 398–400.
[20] S.E. Stephans, T.S. Whittingham, A.J. Douglas, W.D. Lust, B.K. Yamamoto, Substrates of energy metabolism attenuate methamphetamine-induced neurotoxicity in striatum, J. Neurochem. 71 (1998) 613–621.
[21] S.E. Stephans, B.K. Yamamoto, Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux, Synapse 17 (1994) 203–209.
[22] F.B. Weihmuller, S.J. O'Dell, J.F. Marshall, MK-801 protection against methamphetamine-induced striatal dopamine terminal injury is associated with attenuated dopamine overflow, Synapse 11 (1992) 155–163.
[23] R.E. Zigmond, M.A. Schwarzschild, A.R. Rittenhouse, Acute regulation of tyrosine hydroxylase by nerve activity and by neurotransmitters via phosphorylation, Annu. Rev. Neurosci. 12 (1989) 415–461.