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Effect of a binge-like dosing regimen of methamphetamine on dopamine levels and tyrosine hydroxylase expressing neurons in the rat brain

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ABSTRACT

Methamphetamine, an amphetamine derivative, is a powerful psychomotor stimulant and commonly used drug of abuse. This study examined the effect of binge-like methamphetamine (MA) dosing (4 × 4 mg/kg, s.c., 2 h apart) on regional dopamine and dopaminergic metabolite levels in rat brain at a range of early time points after final dose (2–48 h). Body temperature was elevated when measured 2 h after the last dose. MA increased dopamine levels in the frontal cortex 2 and 24 h after the last dose. The dopamine level was also increased in the amygdala at 24 h. No change was observed in the striatum at any time point, but levels of the dopamine metabolite DOPAC were markedly reduced at 24 and 48 h. Tyrosine hydroxylase expression is induced downstream of dopamine activity, and it is the rate limiting enzyme in dopamine synthesis. The effect of MA binge-like dosing on the volume of tyrosine hydroxylase containing cell bodies and the area fraction of tyrosine hydroxylase containing fibres was also assessed. MA increased the area fraction of tyrosine hydroxylase fibres in the frontal cortex and reduced the volume of tyrosine hydroxylase containing cell bodies 2 h after last dose in the ventral tegmental area and the substantia nigra. An increase in cell body volume in the substantia nigra was observed 48 h after treatment. These findings collectively highlight the importance of the dopaminergic system in methamphetamine induced effects, identify the frontal cortex, amygdala and striatum as key regions that undergo early changes in response to binge-like methamphetamine dosing and provide evidence of time-dependent effects on the cell bodies and fibres of tyrosine hydroxylase expressing neurons.

1. Introduction

Methamphetamine (MA), a synthetic derivative of amphetamine, is a very potent psychomotor stimulant and is well known for its high potential for abuse. MA intake by an array of routes of administration results in wide distribution in the periphery and the brain, where it interacts and reverses the function of mono-amine transporters, enhancing synaptic levels of dopamine, noradrenaline and serotonin (Cruickshank and Dyer, 2009). Evidence suggests that the mechanism underlying the psychomotor and neurotoxic properties of amphetamine and MA are similar and they also have similar potency (Han and Gu, 2006; Levi et al., 2012). Using in vivo microdialysis, it has been demonstrated that amphetamine and other amphetamine-like stimulants including MA rapidly increase extracellular levels of dopamine in dopamine-rich brain regions such as the striatum (Zetterstrom et al., 1983; Imperato and Di Chiara, 1984; O'Dell et al., 1991). Depletion of dopamine days after drug exposure is another well-established feature of MA and amphetamine-like drugs of abuse (Dackis and Gold, 1985; Metzger et al., 2000; Matuszewich and Yamamoto, 2004; Quinton and Yamamoto, 2006; Atianjoh et al., 2008), however dopamine tissue levels in the hours immediately following the rapid increase in extracellular levels, within the first 48 h after drug exposure is less widely investigated, and is the subject of the present study.

It has been suggested that low doses of MA affect the CNS by activation of D2 receptors, while high doses additionally activate D1 receptors (Alburges et al., 2001a; Alburges et al., 2001b). Following stimulation of D1-like receptors, the subsequent increase in cAMP and influx of calcium activates protein kinase A, which can phosphorylate cAMP response element binding protein (CREB). It is known that drugs such as amphetamine and cocaine activate D1 receptors and induce phosphorylation of CREB (Dudman et al., 2003). Phosphorylated CREB acts as a transcriptional factor for many downstream genes such as tyrosine hydroxylase (Piech-Dumas and Tank, 1999).

Tyrosine hydroxylase is an enzyme that catalyses the conversion of L-tyrosine to L-DOPA. It is the rate-limiting enzyme in the synthesis of dopamine and noradrenaline. Many studies have shown an alteration in

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tyrosine hydroxylase following MA exposure. For example, a transient increase in tyrosine hydroxylase mRNA has been demonstrated in the rat locus coeruleus following acute MA treatment (Shishido et al., 1997) and an increase in tyrosine hydroxylase protein was observed in the mouse cerebellar cortex 3 days after a binge MA dosing regimen that correlated with the motor dysfunction caused by the drug (Ferrucci et al., 2007). In contrast, chronic use of MA has been shown to cause a decrease in tyrosine hydroxylase levels (Kogan et al., 1976; Di Monte et al., 1996) that may be persistent (Hotchkiss and Gibb, 1980) and post-mortem studies of chronic MA users have shown reduced dopamine levels and tyrosine hydroxylase expression in the striatum (Wilson et al., 1996).

Animal models have shown that single or repeated doses of methamphetamine promotes behavior and biochemical changes. Single large doses of methamphetamine up to 15 mg/kg have been used (Brown et al., 2002), although recent studies(Good and Radcliffe, 2011; Braun et al., 2011) have demonstrated that repeated lower doses reduce risk of mortality and more closely mimics that used by humans. A regimen using 4 injections of methamphetamine at 2 h intervals is the most common approach(Taraska and Finnegan, 1997; Wallace et al., 1999; Pereira et al., 2006).

Dopaminergic fibres project to the striatum (nigrostriatal pathway), the cerebral cortex (mesocortical pathway) and the limbic system (mesolimbic pathway). There is considerable literature on the effects of MA in the striatum, but less information on the mesocortical and mesolimbic target sites. This study investigated the early effect of binge-like MA dosing on dopamine neurotransmitter levels in the frontal cortex and the amygdala, in addition to the striatum, at time points from 2 h after final binge dose, up to 48 h. The effect of MA binge dosing on neurons expressing tyrosine hydroxylase over the same time period was also assessed, specifically the cell body volume of neurons in the substantia nigra and ventral tegmental area, and the area fraction of tyrosine hydroxylase containing fibres in the frontal cortex. The acute hyperthermia triggered by MA has been associated with deficits in the dopaminergic system, oxidative stress and ultimately with neurodegeneration(Krasnova and Cadet, 2009), so it was of interest to correlate the changes in dopaminergic neurons with changes in body temperature to advance understanding of the effect of methamphetamine on dopaminergic neurons.

2. Materials and methods

Sprague Dawley rats (250–350 g) were used in this study. Procedures were carried out under the guidelines of the Animal Welfare Committee of the National University of Ireland, Galway and in accordance with the EU Directive (2010/63/EU).

Rats (n = 6 or 7 per time point) were housed singly and maintained on a 12 h light/dark cycle (lights on at 08:00 h and off at 20:00 h). The housing facility was temperature (20 °C ± 2 °C) and humidity (40–60%) controlled. Food and water were available ad libitum.

(+) Methamphetamine-HCl was purchased under license from Sigma-Aldrich, USA. Rats were administered four doses of MA, 4 mg/kg s.c. (calculated as free base), 2 h apart or saline for control. Repeated dosing of MA at 2 h intervals is commonly used to mimic binge-like drug taking behavior (Braun et al., 2011; Wallace et al., 1999; Pereira et al., 2006). The 4 mg/kg dose used in this study was previously shown to be the highest safe level in our hands (data not shown).

Body temperature was measured in animals treated with methamphetamine and the saline treated controls by rectal thermometer at 2, 24 or 48 h after the last dose, immediately prior to sacrifice and brain dissection for analysis.

Concentrations of dopamine, its metabolites and 1–3-4-dihydroxyphenylamine (L-DOPA) were measured by HPLC with electrochemical detection using the method of (Seyfried et al., 1986). Briefly, each brain was dissected on ice into regions of interest (frontal cortex, amygdala and striatum) and stored at -80 °C until further processing. The fresh frozen brain tissue ($n = 7$ per group) was sonicated in ice cold homogenizing buffer, and the supernatant separated on a Merck Licrosorb RP-C18 column. N-Methyl-5-HT (2 ng/20 µl) was used as the internal standard in all samples. An external standard mix containing 200 ng/20 µl of dopamine, L-DOPA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was run between every 5 samples to recalibrate the system and minimize any drift in amine retention times as sampling proceeded. Peak height data from chromatograms together with data obtained from brain tissue weights and internal and external standard mixtures were used to calculate neurotransmitter concentration in each brain sample in terms of ng/g of fresh tissue.

For immunohistochemistry, animals (n = 6 per group) were deeply anaesthetized with pentobarbital and transcardial perfusion was performed with 4% *v*/v paraformaldehyde. The brains were stored in 20% *w/v* sucrose in PBS with 0.1% w/v sodium azide at 4 °C. Coronal brain sections of 40 µM thickness were cut on a microtome and floated in Trizma Buffered Saline (TBS) with 1% w/v azide solution. Sections were washed with TBS (3 × 5 min), placed in 0.75% *v*/v hydrogen peroxide/methanol in distilled water for 5 min in order to quench the endogenous peroxides in the tissue, followed by further washes in TBS (3 × 5 min). Sections were then blocked with 3% v/v normal horse serum in Triton-X TBS (TXTBS) for 60 min, to avoid unspecific binding. Then sections were incubated for 24 h at room temperature and constant agitation in TXTBS containing tyrosine hydroxylase antibody (mouse, anti-rat; 1:1000 dilution, Vector Laboratories) and 1% *v*/v normal horse serum. After incubation, sections were washed in TBS (3 × 10 min) and incubated for 3 h in TBS containing biotinylated horse anti-mouse secondary antibody (1:200 dilution; Vector Laboratories) in 1% *v*/v normal horse serum. After incubation, sections were rinsed in TBS (3 × 10 min) and incubated in avidin-biotin-peroxidase complex (ABC) in TBS 1% *v*/v normal horse serum for 2 h. Sections were then rinsed in TBS (3 × 10 min) and incubated for 5 min in 0.02% 3,3-diaminobenzidine-4HCl (DAB) and 0.01% v/v H_2O_2 in TBS to reveal immunoreactivity. The reaction was stopped by washing the section in TBS (3 × 5 min). The sections were then mounted on glass-gelatinised slides and air-dried overnight. Sections were then dehydrated in a series of ethyl alcohol (5 min in 50% alcohol, two times 5 min in 70% alcohol and 5 min in 100% alcohol), cleared with xylene (2 × 5 min) and coverslipped with DePex mounting medium.

2.1. Quantification of tyrosine hydroxylase fibres in the frontal cortex

Photomicrographs (20× magnification; five images per slice, six per animal) of the prefrontal cortex (region Fr2, interaural 13.7 mm, bregma +4.7 mm, (Paxinos and Watson, 1986)) were analysed. Tyrosine hydroxylase fibre area was determined using Image J software. For this, images were first converted to gray scale 8-bit images, threshold defined and the area fraction of stained fibres was assessed by dividing by the area of the field of view.

2.2. Estimation of nuclear volume of tyrosine hydroxylase containing neurons in the Substantia Nigra (SN) and Ventral Tegmental Area (VTA)

Photomicrographs (20×; two images from each hemisphere) of the substantia nigra (pars reticulata and pars compacta) and ventral tegmental area were analysed. Volume-weighted mean volume ($\overline{V}v$ Nuc), was estimated as described by (Dockery et al., 1997), and is an estimate of cell body volume. Briefly, this was determined by the point sampled intercept method (Gundersen et al., 1988). A lattice of test points on lines was superimposed randomly onto the cell bodies in each particular field. When a point hit a cell body, a line was drawn through the point from one cell body margin to the other. These lines produced point-sampled intercepts whose length were measured in cm, changed to µm and divided by the magnification of the picture (in this case 500), to give the real size (l_o). The number was then cubed and the mean multiplied by $\pi/3$. The average overall intercepts gave an unbiased