NeuroToxicology 38 (2013) 61–66

Contents lists available at SciVerse ScienceDirect

NeuroToxicology

Amphetamine makes caudate tissue more susceptible to oxygen and glucose deprivation

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ARTICLE INFO

Article history:
Received 15 January 2013
Accepted 7 June 2013
Available online 17 June 2013

Keywords:
Amphetamine
Caudate
Dopamine
Ischaemia
Mitochondria
Voltammetry
TTC

ABSTRACT

Amphetamine is being investigated to reduce morbidity following stroke. However, the medicinal use of amphetamine is complicated because this drug is addictive, cardiotoxic, and can be neurotoxic. Thus, further research into the safety of giving amphetamine to stroke patients is required. Here, we examine whether prior treatment with amphetamine has any effect on oxygen–glucose deprivation (OGD)-evoked transmitter efflux and mitochondrial function. To circumvent the well-documented cardiovascular effects of amphetamine we have used rat brain slices.

Brain slices were exposed to 30 μM of amphetamine for 10 min 1 h before being exposed to OGD. Using fast cyclic voltammetry in rat caudate, dopamine efflux induced by OGD was measured. The effect of amphetamine on mitochondrial function was examined using triphenyltetrazolium chloride (TTC) staining.

Prior amphetamine exposure decreased the time to onset of OGD-evoked dopamine efflux (from 460 to 220 s), suggesting that the caudate was more sensitive to OGD. This increased sensitivity to OGD was attenuated by pre-treatment with the dopamine transporter blocker GBR12909 (1 μM). Pre-treatment with the dopamine D_2 receptor antagonist metoclopramide (1 μM) had no effect on the amphetamine-evoked sensitisation to OGD. Amphetamine decreased TTC staining in the caudate suggesting that amphetamine compromised the dopamine system by disrupting mitochondrial function.

Amphetamine treatment may be harmful in stroke recovery by making the brain more vulnerable to ischaemia. These data also suggest that amphetamine abusers might be more susceptible to cerebral ischaemia.

1. Introduction

There has been much recent interest in reducing stroke-related morbidity using amphetamine-like drugs (Lokk et al., 2011; Sprigg and Bath, 2009). Pre-clinical research has shown that amphetamine can promote neuronal growth, alter neuroplasticity and re-innervate the lesion by altering gene transcription following stroke (Liu et al., 2011). Another study has shown that administering methamphetamine post-stroke can improve neuronal survival and reduce infarct volume by inducing the efflux of dopamine and subsequently activating the PI3K-AKT neuroprotective pathway (Rau et al., 2011). Further, amphetamine can result in neural sprouting and enhanced synaptogenesis (Stroemer et al., 1998) and augmentation of dendritic length and density, perhaps reflecting enhanced synaptic connectivity (Robinson and Kolb, 1997). The use of amphetamine in stroke has been reviewed and although more trials are needed to draw firm conclusions there is evidence of improved motor and language function in amphetamine treated patients (Sprigg and Bath, 2009; Martinsson et al., 2008).

Despite these potential beneficial effects of amphetamine, this drug is known to increase the risk of stroke by inducing hypertension and cardiac arrhythmia (Sprigg and Bath, 2009; Dawson and Moffatt, 2012). In addition, high concentrations of amphetamines and dopamine are known to be neurotoxic (Lancelot et al., 1995; Davidson et al., 2001; Cadet et al., 2007). The aim of this study was to examine the acute effects of amphetamine in an in vitro model of stroke, circumventing cardiovascular effects of amphetamine. To this end, rat caudate brain slices were exposed to amphetamine for 10 min and the effects on oxygen glucose deprivation (OGD) were examined 60 min later.

2. Materials and methods

2.1. Artificial cerebral spinal fluid (aCSF)

Maintenance and ischaemic aCSF both contained (in mM) NaCl (126), KCl (2.0), KH_2PO_4 (1.4), $MgSO_4$ (2.0), $NaHCO_3$ (26.0) and $CaCl_2$ (2.4). However, the ischaemic solution contained only

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http://dx.doi.org/10.1016/j.neuro.2013.06.004

2.0 mM D-glucose and was bubbled with 95% N_2/5% CO_2 while the maintenance solution contained 10 mM D-glucose bubbled with 95% O_2/5% CO_2.

2.2. Brain slices

Caudate brain slices (+2.0 to 0.0 mm versus bregma, Paxinos and Watson, 1986) were obtained from 8-week old male Wistar rats, kept 4 per cage with food and water available ad libitum. Rats were killed by cervical dislocation without anaesthetic. Brains were removed under ice-cold aCSF and 400 μm coronal slices were taken using a vibratome.

2.3. Measurement of mitochondrial activity

Triphenyltetrazolium chloride (TTC) staining was used to measure mitochondrial activity in the caudate after exposure to amphetamine. Brain slices were first incubated in maintenance aCSF for 30 min then either maintenance aCSF or 0.3, 3 or 30 μM amphetamine for 10 min, then maintenance aCSF for a further 60 min. Brain slices were then placed in 0.25% TTC solution for 40 min before fixing using 4% paraformaldehyde for 20 min. A digital image was then taken and analysed using Image J (NIH). For the TTC experiments 4 brain slices from each of 5 rats were exposed to each concentration, that is we used a within subjects design.

2.4. Detection of dopamine efflux

Fast cyclic voltammetry is an electrochemical monitoring technique where we apply a low voltage waveform (−1 to +1.4 V; Fig. 3A) to a carbon fibre electrode at 1 Hz using a Millar voltammeter; any dopamine within the vicinity of the electrode will be oxidised and release 2 electrons. By measuring at the dopamine oxidation potential (600 mV) the Faradaic current can be used to estimate the dopamine concentration after electrode calibration (Fig. 3B). If one subtracts the signal in the presence of 5 μM dopamine, from the signal when no dopamine is present one obtains the dopamine oxidation and reduction peak Fig. 3C). A subtracted voltammogram after the application of 5 μM 5-HT is shown in Fig. 3D. Note that the 5-HT oxidation peak has a shoulder at ~500 mV and that there are 2 small reductions peaks (versus one reduction peak for dopamine). Caudate slices were superfused with normal aCSF and the carbon electrode was placed in the dorso-lateral caudate as previously described (Davidson et al., 2011).

Four measures of dopamine efflux were taken: (1) the time to onset of dopamine efflux after the onset of OGD; (2) the peak dopamine level; (3) the time to peak dopamine level from the start of dopamine efflux and (4) the average rate of change of dopamine during dopamine efflux ($\delta DA/\delta t$) as previously described (Toner and Stamford, 1996).

2.5. Experimental protocol

The slice was first equilibrated in normal aCSF for 30 min at 32.5 ± 0.5 °C, exposed to either 10 min of amphetamine (30 μM) or normal aCSF, then again superfused with normal aCSF for 60 min and finally exposed to OGD for 15 min (Fig. 4A and B). Amphetamine for 10 min has previously been shown to be a long enough application to evoke dopamine through reverse transport in striatal slices

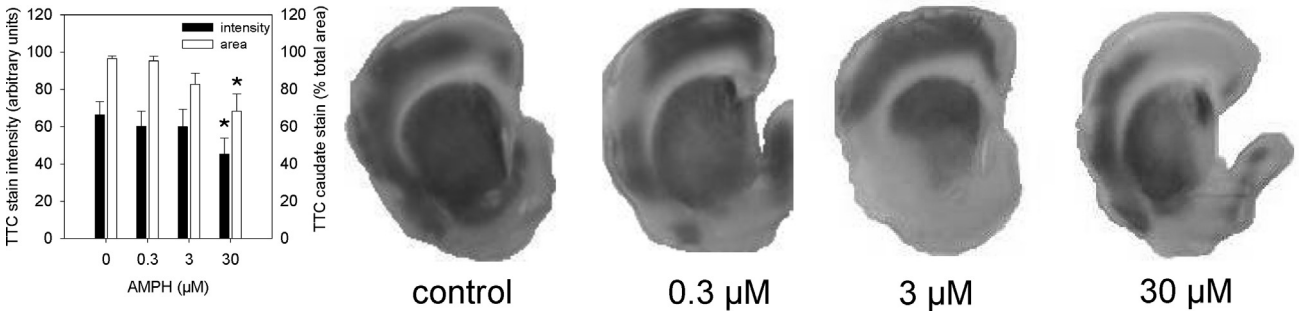

Fig. 1. Mitochondrial function after amphetamine. The effect of amphetamine (0, 0.3, 3 or 30 μM) on the area and intensity of TTC staining of the caudate (left panel). Representative stained control and amphetamine treated slices are shown for a single rat. Values are means ± SEM, $n = 5$ for all groups. $^*p < 0.05$ control versus 30 μM amphetamine treated slices.

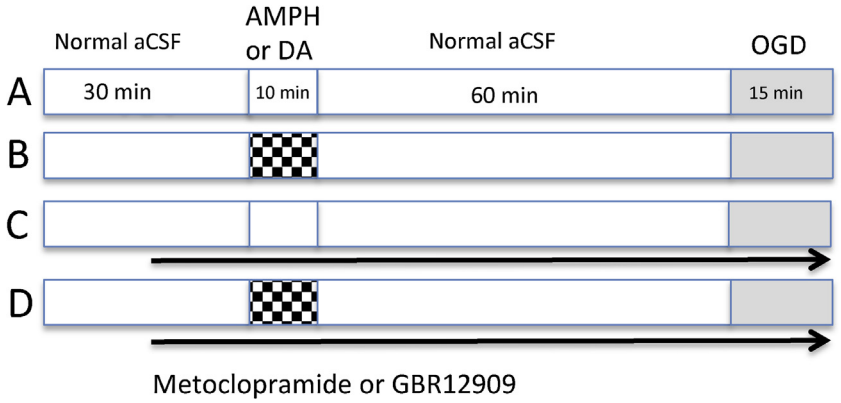

Fig. 2. Protocols for neurochemistry experiments. (A) Control OGD experiments where there was 100 min of normal aCSF then 15 min of OGD. (B) After 30 min of normal aCSF either amphetamine (30 μM) or dopamine (20 or 40 μM) was applied for 10 min. There was then 60 min of normal aCSF and 15 min of OGD as before. (C) After 15 min of normal aCSF either metoclopramide (1 μM) or GBR12909 (1 μM) was applied for the remainder of the experiment (arrow). There was also application of 15 min of OGD as before. aCSF = artificial cerebrospinal fluid; AMPH = amphetamine (black shading); DA = dopamine; OGD = oxygen and glucose deprivation (grey shading).