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COMPARISON OF NORADRENALINE, DOPAMINE AND SEROTONIN IN MEDIATING THE TACHYCARDIC AND THERMOGENIC EFFECTS OF METHAMPHETAMINE IN THE VENTRAL MEDIAL PREFRONTAL CORTEX

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Abstract—Methamphetamine (METH) is a psychostimulant that disrupts monoaminergic neurotransmission to evoke profound behavioral and physiological effects. Rapidly distributing to forebrain regions to increase synaptic concentrations of three monoamines (dopamine (DA), serotonin (5-HT) and noradrenaline (NA)), the medial prefrontal cortex (mPFC) is important in METH-altered behavioral and psychological profiles. Activation of the ventral mPFC can modify physiological variables, however, METH-evoked autonomic changes from this region are unknown. Therefore, the aim of this study was to characterize the respiratory, metabolic and cardiovascular effects of microinjection of METH, DA, 5-HT and NA into the ventral mPFC in urethane-anesthetized Sprague–Dawley rats. METH and NA microinjection evoked dose-related increases in heart rate, interscapular brown adipose tissue temperature and expired CO_2, a pattern of response characteristic of non-shivering thermogenesis. NA and 5-HT microinjection elicited pressor and depressor responses, respectively, with matching baroreflex adjustments in sympathetic nerve activity while METH and DA evoked no change in vasomotor outflow. Low doses of METH and DA may evoke respiratory depression. These data suggest that METH's actions in the ventral mPFC, likely via adrenergic receptors, evoke non-shivering thermogenesis which may contribute to the increased body temperature and tachycardia seen in those that abuse METH.

Key words: thermoregulation, respiration, cardiovascular.

INTRODUCTION

Methamphetamine (METH) is a potent and highly addictive psychostimulant exposing those who abuse it to psychological and physiological dangers. The primary action of METH is to increase both synaptic and cytoplasmic monoamines by blocking and reversing monoaminergic transporters [dopamine (DA), noradrenaline (NA) and serotonin (5-HT) transporters], disrupting monoamine vesicular storage and inhibiting their metabolic breakdown via monoamine oxidase (Brown et al., 2000; Rothman et al., 2001; Sulzer et al., 2005). Due to its highly lipophilic nature, METH distributes into the central nervous system regardless of the route of administration (de la Torre et al., 2004; Mendelson et al., 2006), altering neural circuitry to elicit significant behavioral, psychological and physiological changes (Darke et al., 2008; Cruickshank and Dyer, 2009). The medial prefrontal cortex (mPFC) plays a key role in METH-mediated behavioral and psychological effects (Kalivas and Nakamura, 1999), however, its role in METH-induced physiological changes is unknown.

The physiological effects of METH use include increased heart rate (HR) and arterial pressure (AP), rapid breathing and increased body temperature (Darke et al., 2008; Hart et al., 2008; Schep et al., 2010). These are mediated, at least in part, by the direct actions of METH on sympathetic nerve terminals to increase synaptic NA at peripheral target organs. However, similar physiological effects are seen immediately following central or systemic administration in conscious rodents, in conjunction with increased locomotion (Yoshida et al., 1993; Makisumi et al., 1998; Arora et al., 2001; Sprague et al., 2004; Phelps et al., 2010, Rusyniak et al., 2012).

We have recently reported that disinhibition of the ventral region of the mPFC produced a pattern of autonomic response that included thermogenesis, tachycardia and respiratory stimulation (Hassan et al., 2013). Anatomical evidence indicates that the ventral mPFC innervates multiple autonomic loci (Sesack et al., 1989; Vertes, 2004), and furthermore, the ventral regions of the mPFC are densely innervated by DA, NA and 5-HT-containing terminals (Bjorklund et al., 1978; Carr et al., 1999; Carr and Sesack, 2000; Andrade, 2011).

In order to ascertain whether monoamines in the mPFC drive physiological outflows and contribute to the physical effects evoked by METH administration, our primary objective was to determine the effects of METH,

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Abbreviations: 5-HT, serotonin; AP, arterial pressure; DA, dopamine; HR, heart rate; iBAT, interscapular brown adipose tissue; lSNA, lumbar sympathetic nerve activity; MAP, mean arterial pressure; METH, methamphetamine; mPFC, medial prefrontal cortex; NA, noradrenaline; PNA, phrenic nerve activity; PNamp, phrenic nerve amplitude; PNf, phrenic nerve frequency; SNA, sympathetic nerve activity; sSNA, splanchnic sympathetic nerve activity.

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NA, DA and 5-HT microinjected directly into the ventral region of the mPFC (comprising the ventral prelimbic and infralimbic regions) on respiratory, thermoregulatory and cardiovascular outflows. The natural ligands of DA, 5-HT and NA receptors were chosen to permit synergistic interactions that occur at DA, 5-HT and NA receptors and transporters. The physiological variables measured in this study included phrenic nerve burst amplitude and frequency (respiratory), AP and HR (cardiovascular), splanchnic and lumbar sympathetic nerve activities (cardiovascular and metabolic), expired CO_2 (respiratory and metabolic), and interscapular brown adipose tissue (iBAT) temperature (metabolic).

EXPERIMENTAL PROCEDURES

Ethical approval

All procedures conformed to the regulations detailed in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Macquarie University Animal Care and Ethics Committee.

General Procedures and recordings

Thirty-six male Sprague–Dawley rats weighing 350–550 g (Animal Resource Centre, Perth) were anesthetized with urethane in saline (10% solution administered at 1.3 g kg^{-1} i.p.; Sigma-Aldrich, Australia). Depth of anesthesia was assessed at regular intervals and supplemental urethane was given when necessary (10% solution, 0.2–0.5 ml i.v.). The right femoral artery and vein were cannulated for AP measurement and drug administration, respectively. The rats were intubated to permit artificial ventilation with oxygen-enriched air (Ugo Basile SRL, Varese, Italy). End-tidal CO_2 was continuously monitored and maintained during control conditions at 4–4.5% (Capstar-100, CWE Inc., Ardmore, PA, USA) by adjusting ventilation volume and rate. Colonic (core) temperature was maintained between 36.5–37.5 °C using a thermostatically controlled heating pad (Harvard Apparatus, Holliston, MA, USA). Phrenic nerve activity (PNA), splanchnic (sSNA) and lumbar (lSNA) sympathetic nerve activity were recorded using bipolar silver hook electrodes and bathed in mineral oil. The left phrenic nerve was approached dorsally, isolated and cut. The left greater splanchnic nerve and lumbar sympathetic chain were isolated using a retro-peritoneal approach, as previously described (Burke et al., 2008). The vagi were isolated bilaterally and cut. IBAT temperature was recorded by inserting thermocouples (Physiotemp, NJ, USA) into the left and right iBAT pads (ML312 T-type Pod connected to a PowerLab/30 Series hardware unit via a ML305 Pod Expander; ADInstruments, Australia) as described previously (Hassan et al., 2013). Neuromuscular blockade was maintained with pancuronium bromide (0.8 mg i.v. induction, 0.4 mg h^{-1} i.v. maintenance; Astra Pharmaceuticals Pty Ltd, Sydney, Australia) in a constant infusion of physiological saline (1.5 ml h^{-1}) for hydration. Rats were positioned prone in a stereotaxic frame. Using a flat skull orientation (Paxinos and Watson, 2005), two bore holes were drilled into the skull spanning 2.4–4.0 mm rostral to Bregma and ~3 mm in diameter. The dura was removed for acute microinjection into the ventral mPFC cortex.

Nerve recordings were amplified (×10,000 − ×50,000; CWE Inc., Ardmore, PA, USA), band pass filtered (0.1–3 kHz), sampled at 5 kHz (1401, CED Ltd, Cambridge, UK) and recorded on a computer using Spike2 software (CED Ltd., Cambridge, UK). Core and BAT temperatures and end-tidal CO_2 were sampled at 2 kHz and also recorded using Spike2 software (CED Ltd., Cambridge, UK). HR was derived from AP sampled at 250 Hz. Phrenic nerve frequency (PNf) and phrenic nerve amplitude (PNamp) was derived from PNA.

Experimental protocol

Following surgical preparation, a stabilization period of $\geqslant$ 10 minutes was allowed. Single glass micropipettes were used to pressure inject drugs dissolved in 10 mM phosphate-buffered saline (PBS, pH 7.4) into the ventral mPFC located at + 2.5-3.7 mm rostral, + 0.8 mm medial and 4.0- 5.0 mm ventral from Bregma as identified previously (Paxinos and Watson, 2005; Hassan et al., 2013). Injection volumes were 250 nl. PBS was injected into the region as vehicle controls. Injections of (±)-methamphetamine hydrochloride (METH; Analytical Laboratories, Pymble Australia; 35 nmol $n = 6$, 100 nmol $n = 6$, 335 nmol $n = 7$), dopamine hydrochloride (DA; Sigma Aldrich, Australia; 15 nmol $n = 6$, 53 nmol $n = 5$, 158 nmol $n = 5$), serotonin hydrochloride (5-HT; Sigma Aldrich, Australia;14 nmol $n = 6$, 47 nmol $n = 7$, 141 nmol $n = 5$) or L-norepinephrine hydrochloride (NA; Sigma Aldrich, Australia; 15 nmol $n = 5$, 48 nmol $n = 4$, 145 nmol $n = 5$) were made into the ventral mPFC and autonomic, respiratory and metabolic outflows monitored. In total, 67 injections were made with an average of 2–3 injections per animal (including vehicle) which were randomized for left or right hemisphere and for drug type. At least 40 min was permitted between injections and it was ensured that all responses had returned to baseline levels before proceeding to the contralateral site.

Injection sites were marked with rhodamine labeled microspheres (FluoSpheres, Molecular Probes, OR, USA) or blue fluorescent polymer microspheres (1% Fluoro-Max, Fremont, CA, USA). At the end of each experiment, the rat was euthanized with 3 M KCl i.v. and the cortex removed and placed in fixative overnight (4% formaldehyde in saline). Coronal sections (100 µm) were cut using a vibrating microtome (Leica VT 1000S, Leica Microsystems, Sydney, Australia), mounted on glass slides and cover-slipped for microscopic analysis and confirmation of injection sites.

Data analysis

Nerve recordings were rectified and averaged using a 2-s time constant. Baseline values were obtained by averaging 5 min of data acquired in 60-s bins prior to the microinjection. Peak changes in each variable were determined following drug microinjection with responses expressed as peak value or percentage (%) of baseline activity for each variable; sSNA, lSNA, mean AP (MAP),