Methamphetamine produces bidirectional, concentration-dependent effects on dopamine neuron excitability and dopamine-mediated synaptic currents

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METHAMPHETAMINE IS A PSYCHOMOTOR stimulant that is commonly abused but is also administered clinically at low concentrations to treat attention deficit/hyperactivity disorder, enhance cognition, and decrease hunger. Acutely, methamphetamine-like compounds are commonly used to enhance cognition and to treat attention deficit/hyperactivity disorder, but they also function as positive reinforcers and are self-administered at doses far exceeding clinical relevance. Many of these compounds (including methamphetamine) are substrates for dopamine reuptake transporters, elevating extracellular dopamine by inhibiting uptake and promoting reverse transport. This produces an increase in extracellular dopamine that inhibits dopamine neuron firing through autoreceptor activation and consequently blunts phasic dopamine neurotransmission, an important learning signal. However, these mechanisms do not explain the beneficial behavioral effects observed at clinically useful concentrations. In the present study, we have used patch-clamp electrophysiology in slices of mouse midbrain to show that, surprisingly, low concentrations of methamphetamine actually enhance dopamine neurotransmission and increase dopamine neuron firing through a dopamine transporter-mediated excitatory conductance. Both of these effects are reversed by higher concentrations of methamphetamine, which inhibit firing through dopamine D2 autoreceptor activation and decrease the peak amplitude of dopamine-mediated synaptic currents. These competing, concentration-dependent effects of methamphetamine suggest a mechanistic interplay by which lower concentrations of methamphetamine can overcome autoreceptor-mediated inhibition at the soma to increase phasic dopamine transmission.

G protein-coupled inward rectifier K+ channel; mouse; dopamine transporter; psychostimulant; D2 receptor

MATERIALS AND METHODS

Animals and electrophysiological recordings. Procedures were approved a priori by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio. Male C57Bl6J mice (minimum six-wk old) were maintained and used for all experiments. Mice were anesthetized with isoflurane and killed by de-
capitation. Brains were quickly removed, blocked with a razor blade, and placed on a vibrating microtome for slicing as described previously (Beckstead et al. 2009). Horizontal slices (180–200 μm thick) of the ventral midbrain containing the substantia nigra and ventral tegmental area (VTA) were obtained. Slices were incubated at 34°C for 30–60 min and then maintained at room temperature in a modified Krebs solution containing (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 2.4 CaCl₂, 1.4 NaH₂PO₄, 25 NaHCO₃, 11 d-glucose, and 0.01 MK-801.

For electrophysiological experiments, slices were maintained at 34°C and continuously superfused with 1.5–2.0 ml/min Krebs solution. Dopamine cells in the substantia nigra and VTA were identified visually by size and location in relation to the medial terminal nucleus of the accessory optic tract, the interpeduncular fossa, and the medial lemniscus (Ford et al. 2006). Recordings were divided evenly between the substantia nigra and the VTA, and because no obvious differences were noted between these two brain areas, the data were combined. Further verification of cell identity was provided by examining the neurons’ electrical properties, which included high capacitance, an input resistance, and slow rise times. The firing properties of cells were determined by extracellular recordings and patch clamp experiments (5–10 MΩ) for cell-attached (firing) experiments contained a solution made up almost entirely of Na-HEPES plus 20 mM NaCl, pH 7.40, 290 mosmol/l (Beckstead and Phillips 2009). Dopamine-mediated IPSCs were obtained as described previously (Beckstead et al. 2009). Briefly, a bipolar platinum stimulating electrode (FHC, Bowdoin, ME) was placed a few hundred micrometers caudal to the cell being recorded. A train of five 0.5-ms stimulations was applied at 40 Hz once every 50 s in the presence of the following neurotransmitter receptor blockers: picrotoxin (100 μM, GABA_A), MK-801 (10 μM, NMDA), hexamethonium (100 μM, nicotinic acetylcholine), 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μM, AMPA) and CGP 55845 (100 nM, GABA_B). Other antagonists used at times to block dopamine-sensitive receptors included sulpiride (200–500 nM, D2-type), eticlopride (100 nM, D2-type), SKF 83566 (500 nM, D1-type), and prazosin (100 nM, α₁-adrenergic). Drugs were applied by bath perfusion with the exception of dopamine, which was usually...
applied by iontophoresis (Dagan Instruments, Minneapolis, MN). Dopamine (1 M) was loaded into a high-resistance (~60 MΩ) electrode and placed 10–20 μm caudal to the soma of the cell being recorded. Dopamine was ejected as a cation with a pulse of +150 nA for 2–3 s. Leakage was prevented between pulses by applying a backing current of −25 nA. Because high concentrations of methamphetamine, cocaine, and dopamine incompletely wash out of brain slices, only one cell was recorded per slice.

Drugs. Dopamine hydrochloride, MK-801, DNQX, picrotoxin, hexamethonium, prazosin, eticlopride, fluorocitoxetin, nomifensine, Mg-ATP, Na-GTP, Na-HEPES, K-HEPES, EGTA, and gramicidin D were obtained from Sigma-Aldrich. Isoflurane was purchased from Henry Schein (Melville, NY). CGP 55845, SKF 83566, and GBR 12909 were obtained from Tocris Bioscience (Elisville, MO). BAPTA tetrapotassium salt was obtained from Invitrogen (Carlsbad, CA). Cocaine and methamphetamine (hydrochloride salts) were generous gifts from the National Institute on Drug Abuse drug supply program (Bethesda, MD).

Statistical analyses. One- and two-way analyses of variance (ANOVs) were used to analyze data, and within-cell designs were employed wherever feasible. Tukey’s or Dunnett’s post hoc tests were performed subsequent to significant ANOVAs. Data are presented as means ± SE. In all cases α was set a priori at 0.05.

RESULTS

We sought to investigate the effects of different concentrations of methamphetamine on two distinct components of dopamine neuron physiology: dopamine-mediated synaptic transmission and dopamine neuron firing. We obtained whole cell voltage-clamp recordings (~55 mV) of substantia nigra and VTA dopamine neurons in horizontal slices of mouse midbrain. We first examined the acute effects of methamphetamine on dopamine-mediated IPSCs. As we described previously, dopamine neurons exhibit an electrically evoked slow dopamine IPSC that is mediated through activation of somatodendritic D2 receptors and GIRK channels (Beckstead et al. 2004). Bath perfusion of low concentrations of methamphetamine (0.1–1.0 μM) persistently increased the amplitude of dopamine IPSCs (Fig. 1, A and C), consistent with a decrease in dopamine uptake through DAT. A higher concentration of methamphetamine (10 μM) briefly enhanced IPSC amplitudes as the drug washed into the brain slice and then decreased IPSC amplitudes to slightly below baseline levels (Fig. 1, B and D). Thus methamphetamine exhibited bidirectional effects on dopamine IPSC peak amplitudes (Fig. 1E). In addition, low concentrations of methamphetamine produced a slight increase in the time to peak and half-width of dopamine IPSCs. Higher concentrations of methamphetamine dramatically slowed IPSC kinetics (Fig. 1, F and G).

We next examined the effects of cocaine, which is an abused drug that inhibits DAT but (unlike methamphetamine) is not a DAT substrate. A low concentration of cocaine (0.3 μM) produced similar effects to the low concentration of methamphetamine, enhancing IPSC amplitudes while producing a minor slowing in kinetics (Fig. 2A). However, a high concentration of cocaine (10 μM) produced a greater enhancement of IPSC peak amplitudes while substantially prolonging IPSC kinetics (Fig. 2, B and C). A separate experiment was performed to better mimic the prolonged exposure to psychostimulants that occurs in the brains of drug abusers. Thirty-minute perfusions of either cocaine or methamphetamine (10 μM) indicated that cocaine was more effective than methamphetamine at enhancing the peak amplitude.

Fig. 2. Unlike methamphetamine, high concentrations of cocaine increase dopamine IPSC peak amplitudes. Perfusion of 0.3 μM cocaine [a dopamine transporter (DAT) inhibitor but not a DAT substrate] enhanced the amplitude and slightly prolonged the duration of dopamine IPSCs (A), similar to the effects of 0.3 μM methamphetamine shown in Fig. 1A. However, IPSC amplitudes were further elevated by high concentrations of cocaine (B and C; n = 9–13 cells from 6 mice), whereas the kinetics of the IPSC were substantially prolonged. In a separate experiment, either cocaine or methamphetamine (10 μM) was continuously perfused for 30 min. Summarized data indicate that during prolonged application of methamphetamine, the peak amplitude of dopamine IPSCs decreased (D) to a greater extent than the total charge transferred (area under the curve, E). Differences in the effects of the two psychostimulants were already evident during the first few minutes of perfusion. Error bars are omitted for clarity (n = 4–6 cells from 4–6 mice).
next examined the effects of methamphetamine on basal firing of dopamine neurons. Dopamine neurons in brain slices lack active glutamatergic input and exhibit a slow, tonic pacemaker firing pattern produced by intrinsic conductances. We obtained loose cell-attached recordings of dopamine neurons in slices of mouse midbrain to examine pacemaker firing without disrupting the cell membrane and dialyzing intracellular contents (Fig. 4A). As expected, bath perfusion of high concentrations of methamphetamine (3–10 μM) slowed the firing rate of dopamine neurons, suggesting that methamphetamine increases tonic levels of extracellular dopamine and indirectly activates inhibitory somatodendritic D2 autoreceptors (Fig. 4B). Interestingly, low concentrations of methamphetamine (0.1–0.3 μM) had the opposite effect, producing a subtle and unexpected increase in firing rate (Fig. 4, C and D). We next examined firing in the presence of dopamine receptor antagonists to isolate the excitatory effect of methamphetamine. Under these conditions, methamphetamine produced a concentration-dependent increase in firing that occurred at concentrations an order of magnitude lower than the indirect D2 receptor-mediated inhibition (Fig. 4E).

A DAT-mediated excitatory conductance has been reported previously in expression systems and cell culture, but never before in an ex vivo brain slice preparation (Erreger et al. 2008; Goodwin et al. 2009; Ingram et al. 2002; Sonders et al. 1997). Thus we next tested the hypothesis that the methamphetamine-induced excitation of dopamine neurons in brain slices was due to a DAT-mediated conductance. We examined pacemaker firing of dopamine neurons in the presence of α1-adrenergic and dopamine receptor (D1–D5) blockers. Under these conditions, dopamine neuron firing rate was increased by methamphetamine (10 μM) and was rapidly reversed by bath perfusion of cocaine (10 μM; Fig. 5A). The methamphetamine-induced increase in firing was also blocked by preincubation with the more selective DAT inhibitors GBR 12909 (1 μM) and nomifensine (1–3 μM) but not the serotonin transporter inhibitor fluoxetine (800 nM), suggesting that methamphetamine-induced excitation is mediated through DAT (Fig. 5, B and C). In the presence of the D2-type receptor antagonist eticlopride, firing rate was also increased by the endogenous DAT substrate dopamine, an effect that was also quickly reversed by cocaine (Fig. 5D). We next sought to determine the voltage dependence of the methamphetamine-induced excitation by performing voltage-clamp experiments using the gramicidin perforated-patch technique. At a variety of negative holding voltages, bath perfusion of methamphetamine consistently produced an inward current of ~10 pA that was reversed by cocaine (Fig. 5, E and F). This suggests that the DAT-mediated excitation is not strongly voltage dependent and is not produced by inhibition of a potassium conductance.

**DISCUSSION**

**Acute effects of low concentrations of methamphetamine.**

Here we report that the acute effects of methamphetamine on dopamine neurons are multifaceted and concentration dependent. Low concentrations of methamphetamine directly increasing dopamine neuron firing through a DAT-mediated excitation that occurs independently of dopamine receptor activation. Low concentrations also enhance the amplitude of dopamine-mediated IPSCs. The fact that both of these effects...
Fig. 4. Methamphetamine produces bidirectional, concentration-dependent effects on dopamine neuron firing rate. The rate of dopamine neuron pacemaker firing was monitored using the loose cell-attached patch technique (A). As expected, bath perfusion of high concentrations of methamphetamine (3–10 μM) reduced the firing rate of dopamine neurons, a consequence of increased extracellular concentrations of dopamine producing an activation of somatodendritic D2 autoreceptors (B). Surprisingly, however, lower concentrations of methamphetamine did not inhibit cell firing but actually produced a modest but significant increase in firing rate (C; \( n = 5–15 \) cells from 3–7 mice). D shows the time course of excitatory and inhibitory effects of methamphetamine on firing rate (\( n = 12–15 \) cells from 6–7 mice). To eliminate the contribution of dopamine receptors to the effects of methamphetamine, we repeated the same experiment in the presence of the dopamine D2-type receptor antagonist sulpiride (200–500 nM), the D1/D5 receptor antagonist SKF 83566 (500 nM), and the α1-adrenergic receptor antagonist prazosin (100 nM). Under these conditions, bath perfusion of methamphetamine produced a concentration-dependent increase in pacemaker firing rate that was nearly maximal at 1 μM (E; \( n = 5–11 \) cells from 3–5 mice; 1-way ANOVA followed by Dunnett’s post hoc test: *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \)).

Peak at high nanomolar concentrations suggests that DAT, which has a reported affinity for methamphetamine of 470 nM in mouse (Han and Gu 2006), could be a common molecular target. Low concentrations of methamphetamine do not produce sufficient D2 receptor activation to counteract the increase in pacemaker firing rate produced by the DAT-mediated excitatory conductance. A recently published electrochemical study (Ford et al. 2010) suggests that the time course of dopamine release and the effects of DAT inhibition in the striatum and the VTA are remarkably similar. Thus a methamphetamine-induced increase in firing at the soma could work in synergy with DAT inhibition at the terminal to enhance dopamine-mediated synaptic transmission. This subsequently would enhance the phasic dopamine signals that have been implicated in error prediction and reward learning (Floresco et al. 2003; Phillips et al. 2003; Schultz 2006; Zweifel et al. 2009) and could conceivably explain the cognitive enhancement and increased focus produced by clinical concentrations of DAT substrates. Based on previous pharmacokinetic studies in mice, the low range of methamphetamine doses that cause locomotor stimulation (0.5–1 mg/kg) produce peak brain concentrations of a few micromolar with a half-time of ~1 h (Brien et al. 1978; Kamens et al. 2005; Zombeck et al. 2009). Thus the concentrations of methamphetamine that increase dopamine neuron firing and enhance dopamine-mediated IPSCs are at or below the levels that produce a gross increase in forward locomotion.

Acute effects of high concentrations of methamphetamine. In addition to its effects on DAT, methamphetamine is an intracellular substrate for the vesicular monoamine transporter VMAT2 (Chu et al. 2010; Sulzer et al. 1993). Once inside the cell, methamphetamine displaces vesicular dopamine with a reported IC50 of 13.8 μM in vesicles from rat striatum (Sulzer et al. 2005; Yasumoto et al. 2009). Elevated levels of cytoplasmic dopamine promote reverse transport through DAT (Sulzer et al. 2005), which elevates standing (or tonic) extracellular dopamine levels in both terminal fields and somatodendritic regions (Di Chiara and Imperato 1988; Kalivas and Duffy 1988). Increased extracellular dopamine can activate somatodendritic D2 receptors, inhibiting neuronal activity by activating GIRK channels (Beckstead et al. 2004; Lacey et al. 1987). This mechanism is likely responsible for the decreased dopamine neuron firing we observed at higher methamphetamine concentrations. Pacemaker firing completely ceased in 2 of 11 neurons exposed to 3 μM methamphetamine and in 9 of 15 neurons exposed to 10 μM methamphetamine. Exposure to 10 μM methamphetamine also produced sufficient dopamine release to induce a modest but significant outward current in voltage-clamp experiments.

Although 10 μM methamphetamine appeared to increase tonic levels of dopamine, it inhibited the peak amplitude and slowed the kinetics of electrically evoked dopamine IPSCs. These effects most likely occurred through a combination of three mechanisms. First, sustained activation could desensitize postsynaptic D2 receptors or their G protein-mediated signaling pathway. Second, increased extracellular dopamine could occlude D2 receptor activation. Third, methamphetamine could decrease dopamine vesicular content. The concentration of 10 μM methamphetamine undoubtedly produces occlusion to some extent, as suggested by the outward current observed in voltage-clamp experiments. However, the outward current averaged 17.6 pA, or only 6.5% of the average maximal current produced during the iontophoresis experiment. Exposure to 10 μM cocaine also
produced a comparable outward current (15.7 ± 1.7 pA, n = 7, not shown) but more effectively enhanced IPSC amplitudes, suggesting that occlusion cannot explain the differences between the effects of cocaine and methamphetamine. Furthermore, the iontophoresis experiment indicated that the combined reduction produced by occlusion and desensitization was just 12%. Therefore, it is likely that the decrease in vesicular content produced by methamphetamine is responsible for the disparities between the two psychostimulants. Methamphetamine and similar compounds reduce quantal content of dopamine release, probably by acting as VMAT2 substrates and/or disrupting vesicular pH gradients (Sulzer 2011). This could decrease synaptic dopamine concentrations, slowing the synchronous receptor activation produced by evoked release (Ford et al. 2009) and decreasing IPSC amplitudes to a greater extent than slower currents induced by iontophoresis. A rapid disruption of vesicular content is consistent with the rapid decrease in stimulated synaptic overflow by α-amphetamine reported in striatal brain slices (Jones et al. 1998). Thus, although all three mechanisms probably contribute to the effects of methamphetamine, decreased vesicular dopamine content probably contributes most to the reduction in IPSC amplitude.

A fourth plausible mechanism that we do not believe contributes substantially to our results is the decrease in DAT function and/or surface expression produced by DAT substrates (Gulley and Zahniser 2003; Sandoval et al. 2001). Methamphetamine application in this study was typically limited to 10 min, and we made no subsequent attempt to completely wash methamphetamine out of our slices. Thus any analysis of dopamine clearance would be confounded by the continued presence of the uptake inhibitor and would be uninterpretable. Although we do not know how sensitive DAT-mediated excitation is to changes in surface expres-
sion, there does not appear to be any tachyphylaxis represented in Fig. 5, A and B, but there may be a small bit indicated in Fig. 5E.

DAT-mediated excitation of dopamine neurons. Our findings suggest that methamphetamine produces an excitatory conductance in dopamine neurons that is mediated through DAT. Importantly, this excitation can be observed at concentrations an order of magnitude lower than mass inhibition through D2 receptor activation and thus can be observed even when D2 receptors are not blocked. DAT-mediated excitation may contribute to the effects of low methamphetamine doses that are observed clinically (such as increased focus) or in the early stages of drug abuse (such as euphoria and reinforcement). Previous studies suggest that other DAT substrates can produce similar effects. Seutin et al. (1991) reported in the text, but did not show, that amphetamine increases the firing of dopamine neurons in brain slices in the presence of a D2 receptor antagonist. Data obtained using expression systems and cell culture later identified DAT-mediated excitation produced by dopamine, D-amphetamine, and methamphetamine, including one report in cultured dopamine neurons (Erreger et al. 2008; Goodwin et al. 2009; Ingram et al. 2002; Sonders et al. 1997). In vivo, amphetamine can also excite dopamine neurons through an α-adrenergic receptor-mediated circuit mechanism (Shi et al. 2000, 2007). Like amphetamine, methamphetamine is a substrate for both DAT and VMAT2 and can increase extracellular dopamine concentrations. The two compounds do differ somewhat in their abuse liability as well as their central effects (Goodwin et al. 2009; National Institute on Drug Abuse 2006; Shoblock et al. 2003a, 2003b).

In summary, the acute effects of methamphetamine on dopamine neuron excitability and output are concentration dependent and bidirectional. At low concentrations, methamphetamine increases dopamine neuron firing and enhances stimulated dopamine neurotransmission. At higher concentrations, both effects are reversed. Movement of dopamine out of the vesicles and into the extracellular space slows dopamine neuron firing while decreasing the peak amplitude of electrically evoked dopamine neurotransmission. These observations could explain the therapeutic window produced by low concentrations of methamphetamine. DAT-mediated excitation of dopamine neuron firing could work in concert with enhanced neurotransmission in terminal regions to produce the beneficial behavioral effects (i.e., increased attention and cognition) observed with low doses of DAT substrates.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: S.Y.B. and M.J.B. performed experiments; S.Y.B. and M.J.B. analyzed data; S.Y.B. and M.J.B. interpreted results of experiments; S.Y.B. and M.J.B. drafted manuscript; S.Y.B. and M.J.B. approved final version of manuscript; M.J.B. conception and design of research; M.J.B. prepared figures; M.J.B. edited and revised manuscript.

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